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Bacteriological studies on some Iowa creamery water supplies

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BACTERIOLOGICAL STUDIES ON SOME IOWA CREAMERY WATER SUPPLIES

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TABLE OF CONTENTS

Page

INTRODUCTION

Some of the more serious flavor defects of butter are due to bacterial action. Since bacteria are widely distributed in nature, they may be introduced into butter from a variety of sources. Results of studies on butter defects believed due to protein or fat decomposition by bacteria have suggested that water used in the manufacturing process may be one of the sources of objectionable organisms.

In the manufacture of butter, water is used to wash the butter granules relatively free of buttermilk and is added in composition control. Also, large quantities of water are used in the creamery for cleaning purposes. If butter comes in contact with water and equipment containing undesirable organisms, costly defects in flavor may result. Due to the increasing demand for lightly salted or unsalted butter, the protective action of salt has been reduced or eliminated and defects once rarely encountered have become more common. While salt has an inhibitory effect on most organisms, various factors influence this and serious microbiological defects occasionally occur in salted butter.

Water that is unfit for use as drinking water should not be employed by food-processing plants. However, water considered potable by drinking water standards may not be suitable

for dairy plant use. In the usual public health examination water is studied for the presence of bacteria of intestinal origin that may indicate possible pollution by pathogenic organisms of the same origin. Water not containing these organisms may, however, contain proteolytic and lipolytic bacteria capable of causing serious defects if introduced into butter. Thus from a bacteriological standpoint, public health authorities are primarily interested in one phase of the problem of water supplies, namely their safety, while additional considerations are necessary in quality control studies on butter. In this connection, the question of microbial growth temperatures complicates the picture. Disease-producing organisms commonly find temperatures close to 37°C. most favorable for development. On the other hand, various bacteria causing defects in butter develop better at approximately 21°C. than at 37°C., with many growing fairly well at comparatively low temperatures (0° to 5°C.). Since butter is often transported and stored at these latter temperatures, the problem of determining sources of undesirable organisms and preventing their entrance into butter is important.

STATEMENT OF PROBLEM

The work herein reported involved bacteriological studies on water supplies of a number of Iowa creameries; these included:

(a) isolation and identification of bacteria undesirable from the standpoint of public health standards;

(b) determination of numbers of total, proteolytic and lipolytic bacteria present;

(c) examination for bacteria of importance from standpoint of certain butter defects;

(d) determination of the relationship between bacteriological quality of water and keeping quality of the butter washed with it; and

(e) use of chlorination for elimination of undesirable organisms.

REVIEW OF LITERATURE

In 1890, an anonymous article (14) stressed the importance of the qualities and quantities of water necessary in a creamery and stated that defective butter often was caused by impure water. It was recommended that samples of all water to be used in dairy plants be examined chemically and bacteriologically. The presence or absence of organisms attacking butter fat and curd was to be established. The importance of types of organisms rather than numbers in the examination of water was stressed.

The following year Jean (31) stated that water plays an important part in the dairy industry and stressed the necessity of a plentiful supply of clean water. He believed that slowly-flowing and still water contained more bacteria than spring water since the germs multiplied more rapidly under the influence of favorable weather and temperature. He noted that unclean water could cause rancidity in butter; fortunately, however, all bacteria did not cause defects in butter and the better quality of the water used, the less chance of rancidity in the butter. Jean prophesied that butter defects other than rancidity, whose causes previously had not been known, could possibly have their origin in water.

The occurence of putrid butter caused by Bacillus foetidus lactis, originating from a patron's water supply, was reported by Jensen (32), in 1891. Apparently Gilruth (18), in 1898, was the first to note the presence of Bacterium fluorescens in creamery water. He also stressed the dangers of using impure water to wash butter.

Putrid butter caused by microbial action was described by Eckles (16). Both Bacterium fluorescens liquefaciens and an unidentified bacterium were isolated, the two being considered responsible for the defect. The fact that the former is common in impure water was noted. Orla-Jensen (63) suggested that since Bacillus fluorescens liquefaciens is widespread in water, it probably is introduced into butter from this source. He stated that the organism acted on butter to produce rancidity and that the presence of at least 2.9 per cent salt prevented growth and fat hydrolysis by it.

McKay and Larsen (50) concluded that the water used to wash butter is an important factor in keeping quality since it may contain germs capable of causing butter deterioration. These can be removed or destroyed by filtration or pasteurization, the latter being an economically sound practice. The authors found that the use of pasteurized cream, pasteurized water and salt improved the keeping quality of butter; numerous samples of butter displayed cheesy, rancid and other defects, which were believed due to impure water. They stated that all well water, especially that from shallow wells, con-

tains bacteria and listed numerous opportunities for contamination of shallow wells; they believed deep wells were much superior for creamery water supplies. An instance in which Bacillus fluorescens liquefaciens was found in a water supply was cited.

Melick (52) reported a direct relationship between the bacterial content of the wash water used and the keeping quality of butter. He concluded that sterilization of butter wash water was practical and stated that filters were of no value unless they were frequently cleaned and refilled. Mrozek and Meetz (59) obtained no definite correlation between condition of water supplies, studied bacteriologically and chemically, and quality of butter manufactured.

McKay and Bower (49) found that creamery water often produced objectionable off-flavors in raw, sweet cream butter, while with butter made from pasteurized cream containing butter culture a smaller number of samples were defective. The presence of salt and use of butter culture were considered of value in controlling difficulties in butter manufacture due to poor water supplies. The investigators stated that all water containing bacteria did not necessarily produce bad flavors in butter since the bacteria in question must be able to grow in competition with other bacteria in the presence of salt and lactic acid.

Sayer, Rahn and Farrand (76) noted that some of the bacteria in butter, including Bacillus fluorescens liquefaciens, are derived from the wash water.

Dornic and Daire (15) stated that various organisms, among them Bacillus fluorescens liquefaciens, were capable of producing rancidity in butter. They believed that the more dangerous of these microbes had their origin in water used to wash butter, the water being contaminated by infiltration. They suggested that pasteurization of cream is a wasted process if the butter is washed with water containing bacteria capable of producing rancidity. Studies carried out with ultra-violet rays showed that the sterilization of water by this method was practical.

In New South Wales, Brown (2) observed that Bacillus fluorescens liquefaciens was associated with unsatisfactory water and had a putrefactive action on certain constituents of butter and cream. He stressed the necessity of a thorough clean-up of both farm and factory and added that this organism was found in other types of inferior butter as well as in rancid butter. He believed that cheesiness in butter was caused by contamination on the farm or in the factory.

Various cases in which off-flavors in butter were traced to defective water supplies were cited by MacInnes and Randell (47) of New South Wales. A number of these were found to contain large numbers of undesirable types, including Bacillus fluorescens liquefaciens, lactose-fermenting bacteria, proteolytic bacteria, yeasts and molds. The authors stressed the importance of a good water supply in the manufacture of high-quality butter and advised improvement of supplies where

necessary, including new supplies or new plant sites where no other course was open.

The importance of bacteriologically pure water in creameries has been emphasized by many additional reports (3), (10), (24), (27), (33), (38), (42), (43), (60), (64), (65), (67).

In Canada, the work in relation to creamery water supplies developed as a result of investigations on "surface taint" in butter; this defect is commonly called "rabbito" in Australia and "putrid defect" in the United States and other countries. Sadler and Vollum (74) examined the water supplies of certain creameries having difficulty and found them quite unsatisfactory, with Escherichia-Aerobacter organisms, among others, reaching the butter in considerable numbers. While the causative organism was not determined, the results in general indicated unsatisfactory water supplies. The authors warned that where the water supply is contaminated, the product of a creamery is at all times liable to direct or indirect infection. In one case cited, butter deterioration ceased when the water supply was improved and the storage tank cleaned. Recommendations made included examination of the water supply from time to time, treatment with chlorine or heat, or procurement of a new supply if necessary and thorough cleansing and sterilization of utensils. The presence of Pseudomonas fluorescens in the butter was noted.

Hood and White (26), also in Canada, studied surface taint in butter and concluded that inefficient sanitary control was responsible. The butter samples examined contained many proteolytic organisms, and the total bacterial and yeast counts were high; however, routine examinations of samples from creameries having difficulty often gave both high and low counts. Pseudomonas fluorescens was found in some of the butter samples examined. Certain organisms isolated from well water and inoculated into cream gave the defect in as short a period as 2 days.

The belief that Pseudomonas fluorescens was the organism causing surface taint or surface flavor in butter was advanced by Shutt (78, 79). He reported that city creameries (using pure water) encountered no difficulty, whereas rural creameries (often using impure water) had considerable trouble, especially after long wet periods. He concluded that impure water supplies were responsible for surface taint and stated that water considered safe for human consumption is not necessarily safe for washing butter. Studies in a number of creameries showed the defect could be eliminated when measures for improvement were taken; such control efforts included the substitution of pure water for contaminated water by sinking deep wells, or heating water to 88°C. for 10 minutes and cooling. In the few cases where these measures could not be carried out, neutralization of cream to 0.35 per cent titratable acidity, instead of to 0.25 per cent (the

usual limit), was suggested.

Virtanen (89) stated that cheesy, fermented, putrid and rank defects in butter were caused by enzymes produced by certain water bacteria belonging to the Pseudomonas fluorescens and Pseudomonas punctatum groups.

A number of investigators, including Morgan (56), Löhnis (43), Orla-Jensen (63, 65), Dornic and Daire (15), Stocker (84) and Busch (4) have pointed to the important part played by Pseudomonas fluorescens and its closly related forms in producing rancidity in butter. Stocker (84) warned against the use of contaminated wash water containing Bacterium coli, Bacterium fluorescens and other undesirable types. Busch (4) found Bacterium fluorescens in 13 per cent of the water samples examined and emphasized the importance of continuous vigilance concerning the quality of creamery water supplies. When necessary, control measures were advocated. Busch noted that the quality of the water was especially poor in the spring and fall, (during periods of heavy rains) and that bacterial infections of this nature apparently could arise and disappear of themselves.

The importance of satisfactory water supplies on farms producing butter was shown by Thomas and Morgan (86) in a study of the keeping quality of Welsh butter. They warned that water used to wash butter should be free of undesirable organisms, such as the coliform, fluorescent, spore-forming and proteolytic groups. Of approximately 150 water samples

taken from shallow wells in rural districts producing butter, half were found to contain coliform organisms. Fluorescent bacteria, capable of producing rancidity in butter, also were present in a considerable number of the samples.

Pseudomonas fluorescens was found by Derby and Hammer (13) to cause rancidity in butter. The surface taint defect was, in many cases, believed due to a previously undescribed species, tentatively named Achromobacter putrefaciens and later designated Pseudomonas putrefaciens by Long and Hammer (46). The organism could not be isolated from all samples of putrid butter. The possible relationship of the organism to polluted water was suggested.

Rumment (73) studied the numbers of organisms from wash water that were retained in butter and the effect of this on keeping quality. He stated that since Bacillus fluorescens liquefaciens is a usual inhabitant of polluted water and causes rancidity in butter, its use in his studies was of considerable value. The studies revealed that smaller numbers of organisms were retained in the butter with greater firmness and size of the butter granules.

Meyer (54) described a case wherein, due to a water shortage, the condenser water in the refrigeration system was used as butter wash water. Since the water was known to contain coliform organisms, it was purified, using chlorine in the form of chloride of lime (0.5 ppm.) with satisfactory results. Recommendations made included the covering of water

storage tanks as protection against infection and dirt, preferably with metal plates rather than wood.

In studies on cheesy butter by Herreid, Macy and Combs (25), mixed cultures from defective butter reproduced cheesy defects when inoculated into the wash water. Creamery water supplies sometimes were found to be contaminated with bacteria capable of producing cheddar cheese and other cheesy flavors in unsalted butter. The experience of one creamery that had been troubled with cheesy and putrid flavors in butter over a period of three years was cited. Investigation showed that infected water was the source of the trouble; the difficulty disappeared when a new and satisfactory water supply was provided.

According to Stocker (85), the types and numbers of bacteria occurring in water are dependent to a great extent on the organic constituents present in the water, the chemical character of the water and its temperature, as well as the kind of soil and type of well from which the water came. He noted various methods for determination of undesirable types of organisms and stated that creamery water, as well as drinking water, should not contain more than 50 organisms per ml. Filtration methods were declared preferable to heating methods for reduction of numbers of bacteria in creamery water supplies.

A "musty flavor" defect occurring in New South Wales butter was studied by Randell (70) and found due to the action

of a previously undescribed species of Achromobacter. Although the presence of the organism was not demonstrated in water, satisfactory results in control of the defect were obtained when factory sanitation was improved and precautions taken to use water of known purity in the manufacturing process.

Olson (61) studied bacterial counts and keeping quality of butter made using artifically contaminated water filtered through a Seitz filter. Bacterial counts of butter washed with filtered and unfiltered water were not appreciably different but significant differences in keeping quality were noted in favor of the butter washed with filtered water.

Measures suggested by Sproule and Hamilton (81) for control of surface flavor in butter included chlorination or filtration of all water used in the creamery.

Cullity and Griffin (8) found that outbreaks of rabbito encountered by various factories could be traced in every case to defective water supplies; with the substitution of improved water sources, the trouble disappeared. Two cultures apparently similar to Achromobacter putrefaciens of Derby and Hammer were isolated from water supplies. They concluded tentatively that the causative organism probably was waterborne and that foci of contamination were built up in churns and other equipment as a result of initial contamination from water. High acidity at churning, high salt content and thorough working appeared to retard development of the defect.

The work of Loftus-Hills, Sharp and Searle was cited by Cullity and Griffin (8); organisms believed to be Achromobacter putrefaciens were isolated from factory water supplies, churns, and raw and pasteurized cream, with water supplies considered as the natural habitat.

Discussing dairy water supplies, McLachlan (51) recommended that water for use in butter manufacture should not contain more than 4 bacteria per ml. on plates incubated at 37°C. or more than 15 per ml. on plates incubated at 22°C. He added that Bacterium coli should be absent from 150 ml. of water and that a close examination as to the flora present should be made.

Claydon and Hammer (6) found that wash water inoculated with Pseudomonas mephitica produced a typical skunk-like odor in experimental unsalted butter in 2 to 3 days at 21°C.

Rice (71) suggested that since pasteurization probably kills coliform bacteria present in cream, their presence in butter indicates use of unsterile utensils and impure wash water.

Examination of creamery water supplies by Claydon and Hammer (7) suggested that water used by commercial plants sometimes was capable of causing various defects, including the putrid defect, when used for washing butter. In one case, Achromobacter putrefaciens was isolated from the water supply of a plant having difficulty with this defect. The putrid defect could be produced in unsalted butter when Achromobacter

putrefaciens was added to cream or wash water in such small numbers as to make reisolation difficult. These investigators stated that the wash water which remains in butter is probably in relatively large droplets thus giving organisms present a better chance for extensive development.

Hammer (22) considered equipment and wash water as the two principal sources of Achromobacter putrefaciens. He added that water also contains other organisms producing various defects in butter, including cheesiness and rancidity. For control of defects of this type, adequate pasteurization of cream, care of equipment and materials and satisfactory water supplies were advised. He warned that the latter may undergo sudden unexplained changes, seasonal or otherwise, and that the subject of chlorination of water supplies requires further study, especially concerning the tolerance of various organisms to chlorine.

The possibility that water may meet public health requirements and still be bacteriologically undesirable for creamery purposes has been noted by a number of investigators, including Hammer (22), Turgasen (87), Linneboe (41), Moore (55) and Weckel (90).

Turgasen (87) stated that a significant number of outbreaks of butter spoilage have been traced directly to impure water supplies used for washing butter or for other purposes in the plant. The appearance of the causative organisms in a water supply was sporadic, with a logical ex-

planation for either their appearance or disappearance frequently lacking. They often persisted for long periods. Some of the organisms encountered appeared to be highly resistant to chlorine but adequate chlorination of all water used in the plant was effective in controlling cheesy defects. The author stressed that certain factors in the use of chlorine must be considered, including pH of the water, the period of contact, presence of organic matter and chlorine tolerance of the causative organisms.

The water supplies of 52 creameries in Alberta having difficulty with surface taint butter were studied by Linneboe (41). With the 200 water samples examined, Pseudomonas putrefaciens was present in 9 well samples and in 5 additional samples from holding tanks. Six of 55 farm water samples contained the organism. An example showing that the ordinary public health analysis may not necessarily indicate the suitability of water for creamery purposes was cited. The well furnishing the water in question was 237 feet deep, was drilled through clay and limestone, with the casing well above ground and set in cement. Samples from the well had been reported as satisfactory, with organisms of the Escherichia-Aerobacter group absent and with low counts on plating. However, considerable difficulty with surface taint butter had been experienced by the plant and Linneboe isolated Pseudomonas putrefaciens from the water supply. He concluded that pasteurization of cream and a pure water supply

are necessary in the control of the surface taint defect.

Sorensen (80) examined samples from 22,060 churnings of commercial butter and visited the creameries producing the butter of poor keeping quality. Putrid and cheesy defects were the ones most frequently encountered; the major cause of the defects was found to be contaminated water. Rancid flavors frequently were traced to equipment in poor sanitary condition. Defective keeping quality due to contaminated water was eliminated by chlorine treatment using hypochlorites.

The work of Morrison and Hammer (57) on the distribution of Pseudomonas fragi showed that this organism, important as a cause of flavor defects in butter because it is proteolytic, lipolytic and psychrophilic, may be present in creamery water supplies. Water samples from 31 creameries were examined and 2 yielded the organism.

Detailed studies by Long and Hammer (46) showed that Pseudomonas putrefaciens is widely distributed in water, including streams, lakes, roadside pools and creamery supplies. Water from 29 creameries was examined, and samples from 14 of the plants yielded Pseudomonas putrefaciens after enrichment in litmus milk. In 8 of the 29 plants the water supplies were suspected as the cause of difficulty with the keeping quality of the butter; Pseudomonas putrefaciens was isolated from water samples from 5 of these 8 plants. A majority of

the samples yielding the organism came from private wells, although in a few cases city water supplies were concerned. The authors concluded that water and plant equipment are of greatest importance in explaining outbreaks of putrid butter.

Itzerott (28) stated that rabbito organisms enter the factory in the cream or water supplies and set up a deep-seated focus of infection in equipment, such as churns. Bacterial counts on butter were found to rise suddenly to high levels just before an outbreak. Recommended control measures included close grading of cream, proper pasteurization, clean equipment with special care given to churns and satisfactory water supplies. Treatment of water with 1 ppm. residual chlorine was advised, especially when the supply was drawn from streams or wells.

Wolochow, Thornton and Hood (91) reported bacteriological analyses on 85 water samples from 37 Alberta creameries, including wells, tanks and city supplies. Potable water often was found to contain relatively large numbers of proteolytic and other types of bacteria capable of growing at low temperatures, with detrimental effects if introduced into butter. Pseudomonas fluorescens was a very common contaminant. The authors suggested that, instead of setting up standards and standard methods of analysis, a more simple attack on the problem of poor creamery water supplies would be bactericidal treatment of all water used in the plants. Future studies could then be concentrated on disinfection

methods.

Of 260 well water samples from rural Ontario examined by Castell and Garrand (5), approximately 10 per cent contained bacteria considered to be of intestinal origin and 30 per cent showed the presence of butyric acid-forming anaerobes. Since samples were not iced, greater significance was attached to types than to numbers of organisms in the samples. It was found that many plates at 2° to 3°C. gave a Pseudomonas fragi odor, while at 25°C. most displayed the odor typical of Pseudomonas fluorescens. Where water was found unfit for human consumption, total counts were high and there were large numbers of undesirable bacteria, suggesting that water unfit for drinking purposes, apart from the sanitary standpoint, is also unfit for dairy purposes. Of water samples "free from intestinal pollution and fit for human consumption", 85 per cent contained proteolytic and lipolytic organisms capable of growing within 4° or 5°C. of freezing. The majority of these were oxidase-positive, a characteristic common to many types of bacteria causing off-flavors in butter.

A review of the literature on rabbito and the allied taints of butter was presented by Pont (68). Although he regarded the direct evidence as far from conclusive, he stated that the original source of infection in butter factories appeared to be the water supplies. Secondary and, in many cases, more serious foci of infection were then set up in

churns and equipment, wooden articles being especially important in this respect. Control measures listed included purification of water supplies by chlorination or filtration and elimination of secondary sources of infection. Pont concluded that the defects were largely the outcome of conditions of production which failed to meet the higher standards demanded by the type of butter manufactured today.

Provan (69) stated that farm and dairy water supplies should equal domestic supplies in quality and in addition should not contain organisms capable of causing quality deterioration in dairy products. He recommended periodic examination of water supplies, basing judgement on these rather than on a few samples taken at random. To determine suitability for domestic usage, he advised total colony counts at 22°C. and at 37°C. and determination of approximate numbers of organisms of the Escherichia-Aerobacter type, with a follow-up for fecal types. Suitability for dairy use would be determined by appropriate technics, including addition of 50,10 and 1 ml. quantities of water to milk or plating of water with subsequent selection of colonies and inoculation into milk. Provan suggested that water with a count in the hundreds per ml. at 22°C. should be regarded with suspicion and should be more thoroughly examined. Where counts are found to fluctuate widely, in accordance with rainfall, the probability is that surface pollution exists. According to Provan, water supplies may be improved by a number of methods,

including protection from the source of pollution, purification of the existing supply and, where necessary, procurement of a new supply. He asserted that although chlorination is the most simple method of control, it requires technical knowledge and aid and often is more costly than other measures, such as removal of the cause of pollution by sealing cracked concrete, and repair of pumps.

Jamieson (30) found that poor water could lower butter quality; however, churn sanitation appeared to exert a greater influence on flavor than did water. Skill in manufacture overcame some defects due to contaminated water and poor churn sanitation. The presence of fluorescent, gelatin-liquifying or other objectionable types was detrimental to butter flavor; the majority of fluorescent and oxidase-positive bacteria were more deleterious to flavor than other types indistinguishable from these by colony growth. Pseudomonas putrefaciens was isolated from 14 of 53 well water samples examined although the characteristic defect caused by this organism developed in only two lots of butter washed with the water samples. He concluded that the presence of surface taint organisms in water, churns or butter did not correlate regularly with the flavor in butter.

The condition known to Australian butter graders as "sectional contamination" was found by Jensen (34) to occur in hand-packed salted butter as a result of the localized action of microorganisms. These were carried onto the sur-

faces of the butter by the utensils and water used in the packing operations. Off-flavors encountered in the affected areas of butter varied from staleness to distinct fruitiness, cheesiness or rancidity, depending somewhat on the age of the butter. Bleaching along the seams often occurred. The primary source of the organisms responsible for the defect was believed to be the water supply. A secondary source of contamination was created by organisms developing in the wood of the utensils used. Chlorination of the water supply and an intensive heat treatment of the wooden utensils resulted in an immediate and complete disappearance of the defect from the butter.

A number of investigators have studied specifically various methods designed to reduce or eliminate the organisms present in butter wash water. Ultra-violet light was studied by Dornic and Daire (15) and Daire (9) and found to be of practical value. Salmon (75) recommended the use of ozone for water sterilization. The katadyn process was investigated by Grimmer and Grenz (19), Demeter and Haase (12) and by Mossel (58). It was not particularly valuable for treatment of butter wash water. The Virtanen alkalization method was studied by Kirkegaard (35), Virtanen (88), Orla-Jensen and Sivertsen (66) and Schäffler (77). When used alone the process was not entirely satisfactory.

The value of filtration, heating and chlorination methods was investigated by Melick (52), Knudsen (36),

Kirkegaard (35), Gross and Hindrikson (21) and Grimmer and Grenz (20). Filtration required constant supervision, with no guarantee of water sterility, pasteurization was expensive and time-consuming while chlorination appeared to give the most satisfactory results. Chlorination was used by Sorensen (80) in commercial practice, with good results. Various methods of water sterilization suitable for creamery use have been reviewed by Knudsen (36), Damm, et al. (10) and Demeter (11).

METHODS

Collection and Shipment of Samples

The samples of water were collected in sterile quart bottles with suitable care by Dr. D. F. Breazeale of Iowa State Brand Creameries, Inc., Mason City Iowa. The iced bottles were shipped in an especially constructed wooden box by Railway Express from Mason City on the evening of collection. On arrival in Ames the following morning, their examination was immediately begun. At plants where samples were collected, general information on the water supply was obtained, such as type, depth and protection of well and whether or not a storage tank was used.

Methods of Bacteriological Examination

The presence of Escherichia-Aerobacter organisms in a sample of water was determined by addition of single 50 ml. and 10 ml. portions of water to equal amounts of double-concentration standard lactose broth and single 1 ml. portions of water to 10 ml. amounts of single-concentration standard lactose broth. The procedure was duplicated with each sample using the tryptose lauryl-sulfate broth of Mallmann and Darby (48) in place of standard lactose broth. To all inoculated

tubes showing liberation of gas at the end of 24 and 48 hours, the "completed test", as outlined in "Standard Methods for the Examination of Water and Sewage" (83), was applied.

Tests used in identification studies on cultures of Escherichia-Aerobacter organisms included determination of (a) indol production, citrate utilization and growth in various carbohydrate bouillons as outlined in "standard methods" (83); (b) hydrogen sulfide production as outlined by Levine, Epstein and Vaughn (40) and (c) production of acetylmethylcarbinol at 30°C. by the modified Voges-Proskauer test recommended by Levine (39). The classification of organisms in Bergey's Manual of Determinative Bacteriology, 5th Edition (1) was followed in attempts to identify cultures isolated.

The numbers of bacteria present in a sample of water were determined by use of nutrient agar prescribed in standard methods (83) and by use of tryptone-glucose-extract agar, listed in "Standard Methods for the Examination of Dairy Products", 8th Edition (82), to which had been added sterile skimmilk (5 per cent) and fat emulsion so that proteolytic and lipolytic organisms could be detected; the fat emulsion was prepared according to the method of Long and Hammer (44) and the natural fat technique suggested by these investigators was employed for detection of lipolysis. The medium is hereafter referred to as the T.G.E.M. agar. Total counts on nutrient agar at 37°C. were made after 24 and 48 hours, the former being considered in the discussions, and on T.G.E.M.

agar at 37°C. after 24 and 48 hours and at 21°C. after 48 and 96 hours, with the 21°C., 96 hour count being considered in the discussion.

Isolation of Pseudomonas putrefaciens was carried out on the gelatin agar suggested by Long and Hammer (46). Following inoculation of 2 ml. portions of water into tubes of litmus milk and incubation at approximately 5°C. for 2 and 4 weeks, small portions of the milk were smeared on plates poured with the gelatin agar medium. These were incubated at room temperature for about 6 days and were closely examined each day. Colonies suggesting Ps. putrefaciens were picked, purified by repeating smearing on gelatin agar plates and eventually identified.

Churning Methods for Keeping Quality Tests

For experimental churnings sweet cream of good flavor was pasteurized at 85° to 90°C. for about 30 minutes. After cooling, portions of approximately 1 pint each were added to quart jars. Churning was carried out in an experimental churn having compartments for holding six jars and a motor which agitated the cream by rotation of a shaft supporting the compartments. The butter granules from each jar were washed with an individual water sample and worked with a paddle in an enamel bowl. When part was to be salted the butter was divided into two portions, one portion remaining unsalted and 1.5 per cent salt being worked into the other.

All portions of butter were placed in small glass jars and held at 21°C. for 7 days. In some cases, following 2 days holding, approximately one-half of each portion was reworked and held in a separate glass jar at 21°C. All equipment, wash water used on control butter and salt were sterilized by autoclaving.

The experimental butter was examined daily for flavor defects.

Interpretation of Keeping Quality Tests

The interpretation of the keeping quality tests on butter washed with the samples of water and held without salt at 21°C. for 7 days requires special care. Relatively few mold spores gaining entrance to such butter from the air can cause off-flavors or even conspicuous areas of mold growth under the holding conditions employed, and the handling of the butter during working, salting, packing, etc. gives ample opportunity for such entrance.

Defects which were not evident in the unsalted butter at 21°C. within 4 days were not considered significant from the standpoint of the quality of water used to wash the butter; also, a musty flavor at 3 days was not considered significant because molds from the air rather quickly produce mustiness in unsalted butter at 21°C.

RESULTS

Data on Samples of Water from Individual Plants

The data on the individual samples of water are grouped on the basis of the plants from which they came and are presented in tables 1 to 70, inclusive.

Plant 1

At plant 1 there are two wells. Normally, water from well A is used, but in periods of shortage water from well B is employed.

The first two of the four samples of water from well A were satisfactory, while the last two were unsatisfactory (table 1). With the satisfactory samples coliform organisms were absent. Total bacterial counts were 3 and less than 1 per ml. on nutrient agar and 1 and 10 per ml. on T.G.E.M. agar. Proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the first sample showed a musty flavor at 4 days, while similar butter washed with the second sample showed no noticeable flavor deterioration at 7 days. The unsatisfactory samples contained no coliform organisms. Total bacterial counts did not exceed 1 per ml. on nutrient

Table 1

Results on samples of water from plant 1

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	[b]well	1-29-41	+ 50 ml.	6	18	1	<1	-	O.K. 7 da.
2.	[a]well	1-29-41	-	3	1	<1	<1	-	musty 4 da.
3.	[b]well	5-27-41	+ 50,10 ml.	1	20	4	4	-	off 3 da.
4.	[a]well	5-27-41	-	<1	10	2	2	-	O.K. 7 da.
5.	[b]well	11-25-41	+ 50,10 ml.	22	700	25	<1	-	unclean 3 da.
6.	[a]well	11-25-41	-	1	180	2	<1	-	O.K. 7 da.
7.	[a]well	11-25-41	-	<1	160	<1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
[a]well = well A
[b]well = well B

agar but were 180 and 160 per ml. on T.G.E.M. agar; proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the two samples there was no noticeable flavor deterioration at 7 days.

The three samples of water from well B were unsatisfactory. Coliform organisms were present in the 50 ml. portion of each sample and were present in the 10 ml. portion of the second and third samples. On nutrient agar total bacterial counts ranged from 1 to 22 per ml., while on T.G.E.M. agar they varied from 18 to 700 per ml. Numbers of proteolytic organisms ranged from 1 to 25 per ml., while numbers of lipolytic organisms varied from less than 1 to 4 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the first sample showed no noticeable flavor deterioration at 7 days; similar butter washed with the second and third samples showed serious flavor deterioration at 3 days in each case.

Plant 2

The three samples of water from the well at plant 2 were satisfactory (table 2). Coliform organisms were absent. On nutrient agar total bacterial counts ranged from less than 1 to 2 per ml., while on T.G.E.M. agar they varied from 2 to 5 per ml. Numbers of proteolytic and lipolytic organisms did not exceed 1 per ml. and Ps. putrefaciens was not

Table 2

Results on samples of water from plant 2

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-2-41	-	2	3	1	1	-	putrid 6 da.
2.	"tank	1-2-41	-	<1	4	1	2	-	O.K. 7 da.
3.	well	4-30-41	-	<1	2	<1	1	-	O.K. 7 da.
4.	tank	4-30-41	-	2	2	<1	1	-	O.K. 7 da.
5.	well	12-2-41	-	<1	5	1	<1	-	O.K. 7 da.
6.	tank	12-2-41	-	200	450	120	<1	-	unclean 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

isolated. Experimental unsalted butter washed with the water showed no noticeable flavor deterioration at 4 days and in two instances was satisfactory at 7 days.

Three samples of water that had been through a wooden storage tank were examined. The first two were satisfactory while the third was unsatisfactory. With the satisfactory samples coliform organisms were absent, total bacterial counts did not exceed 2 per ml. on nutrient agar and were 4 and 2 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 7 days. The unsatisfactory sample showed no coliform organisms, but the total counts were 200 per ml. on nutrient agar and 450 per ml. on T.G.E.M. agar and the proteolytic count was 120 per ml.; the lipolytic count was less than 1 per ml. and Ps. putrefaciens was not detected. In experimental unsalted butter washed with the sample there was no significant flavor deterioration at 4 days; an unclean flavor had developed at 7 days.

Plant 3

At plant 3 water from the well is commonly used; occasionally, water from the city mains is employed.

The first two of three samples of water from the well were satisfactory while the third was unsatisfactory (table 3). With the satisfactory samples coliform organisms

Table 3

Results on samples of water from plant 3

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-19-41	-	1	4	<1	1	-	O.K. 7 da.
2.	well	4-16-41	-	<1	7	2	<1	-	O.K. 7 da.
3.	city	4-16-41	-	<1	600	10	5	-	rancid 5 da.
4.	well	10-20-41	-	<1	1000	25	<1	-	O.K. 7 da.
5.	"tank	10-20-41	-	1	2200	10	<1	-	musty 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = small pressure tank

were absent, total bacterial counts did not exceed 1 per ml. on nutrient agar and were 4 and 7 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the two samples there was no noticeable flavor deterioration at 7 days. The unsatisfactory sample contained no coliform organisms and the total bacterial count on nutrient agar was 1 per ml. but on T.G.E.M. agar the total count was 1000 per ml.; the proteolytic count was 25 per ml., the lipolytic count was less than 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed no evident flavor deterioration at 7 days.

A sample of well water that had been through a small pressure tank was unsatisfactory. No coliform organisms were detected. The total bacterial count on nutrient agar was 1 per ml. but on T.G.E.M. agar it was 2200 per ml.; there were 10 proteolytic and less than 1 lipolytic organisms per ml. and Ps. putrefaciens was not detected. Experimental unsalted butter washed with the sample showed a musty flavor at 4 days.

The one sample of city water from plant 3 was unsatisfactory. Coliform organisms were absent, and the total bacterial count on nutrient agar was less than 1 per ml. but on T.G.E.M. agar it was 600 per ml. There were 10 proteolytic and 5 lipolytic organisms per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with this

sample showed no noticeable flavor deterioration at 4 days but had developed a rancid flavor at 5 days.

Plant 4

At plant 4 water from the well usually is employed; at times water from the city mains is used.

The three samples of water from the well at plant 4 were satisfactory on the basis of general bacteriological data (table 4) but one was unsatisfactory on the basis of its action on butter. With the three samples coliform organisms were absent. Total bacterial counts were each less than 1 per ml. on nutrient agar and were 2, 11 and 2 on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the first and third samples showed no noticeable flavor deterioration at 7 days. Similar butter washed with the second sample was rancid at 4 days; the T.G.E.M. agar plates poured with this sample showed fluorescent organisms which undoubtedly caused the rancidity.

The first of the two samples of water that had been through a metal storage tank was satisfactory while the second was unsatisfactory. With the satisfactory sample coliform organisms were absent, total bacterial counts were 5 and 8 per ml. on nutrient agar and on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with

Table 4

Results on samples of water from plant 4

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	11-27-40	-	<1	2	<1	<1	-	O.K. 7 da.
2.	"tank	11-27-40	-	5	8	1	<1	-	musty 6 da.
3.	city	4-9-41	-	<1	3	2	1	-	O.K. 7 da.
4.	well	4-9-41	-	<1	11	2	2	-	rancid 4 da.
5.	well	1-20-42	-	<1	2	<1	<1	-	O.K. 7 da.
6.	tank	1-20-42	-	26	275	2	<1	-	rancid 7 da.
7.	city	1-20-42	-	1	40	1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

the sample there was no noticeable flavor deterioration at 4 days but a musty flavor had developed at 6 days. The unsatisfactory sample did not contain coliform organisms but total bacterial counts were 26 per ml. on nutrient agar and 275 per ml. on T.G.E.M. agar. Proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed no noticeable flavor deterioration at 4 days while at 7 days a rancid flavor was evident.

The two samples of city water collected at the plant were satisfactory. Coliform organisms were absent. Total bacterial counts on nutrient agar did not exceed 1 per ml., those on T.G.E.M. agar were 3 and 40 per ml., proteolytic counts were less than 1 and 2 per ml. and lipolytic counts did not exceed 1 per ml. Ps. putrefaciens was not detected. Experimental unsalted butter washed with the water displayed no noticeable flavor deterioration even at 7 days.

Plant 5

The three samples of water from the well at plant 5 were unsatisfactory (table 5). Coliform organisms were absent. On nutrient agar total bacterial counts ranged from less than 1 to 11 per ml., but on T.G.E.M. agar they varied from 100 to 3500 per ml.; numbers of proteolytic organisms ranged from 6 to 400 per ml. while numbers of lipolytic organisms varied from 1 to 45 per ml. Ps. putrefaciens was not isolated.

Table 5

Results on samples of water from plant 5

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-2-41	-	11	1000	300	10	-	cheesy 6 da.
2.	"tank	1-2-41	-	15	1000	300	10	+	putrid 4 da.
3.	well	4-9-41	-	<1	100	6	1	-	rancid 6 da.
4.	tank	4-9-41	-	1	160	10	16	-	cheesy 2 da.
5.	well	10-28-41	-	<1	3500	400	45	-	unclean 5 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 4 days; however, serious flavor defects were evident at 6, 6 and 5 days.

The two samples of water that had been through a wooden storage tank were unsatisfactory. Coliform organisms were absent. Total bacterial counts on nutrient agar were 15 and 1 per ml. but on T.G.E.M. agar they were 1000 and 160 per ml. Proteolytic counts were 10 and 300 per ml. while lipolytic counts were 16 and 10 per ml. Ps. putrefaciens was isolated from the first of the two samples. In experimental unsalted butter washed with the samples there was serious flavor deterioration at 4 and 2 days.

Plant 6

The four samples of water from the well at plant 6 were satisfactory on the basis of general bacteriological condition but one sample produced serious deterioration in butter (table 6). With the samples coliform organisms were not detected. On nutrient agar total bacterial counts did not exceed 2 per ml., while on T.G.E.M. agar the total counts varied from 2 to 46 per ml. Proteolytic counts did not exceed 1 per ml., lipolytic counts ranged from less than 1 to 3 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the first, third and fourth samples showed no noticeable flavor deterioration at 7 days. Similar butter washed with the second sample showed a putrid

Table 6

Results on samples of water from plant 6

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	12-4-40	-	2	35	<1	1	-	O.K. 7 da.
2.	"tank	12-4-40	+ 50,10 ml.	<1	250	7	3	-	O.K. 7 da.
3.	well	4-9-41	-	1	4	1	3	-	putrid 4 da.
4.	tank	4-9-41	+ 50 ml.	2	200	13	8	-	off 4 da.
5.	well	10-28-41	-	2	46	1	1	-	O.K. 7 da.
6.	tank	10-28-41	+ 50 ml.	130	290	14	4	-	O.K. 7 da.
7.	well	4-21-42	-	1	2	<1	<1	-	O.K. 7 da.
8.	tank	4-21-42	-	11	140	4	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

condition at 4 days.

The four samples of water that had been through a wooden storage tank were unsatisfactory. Coliform organisms were present in the 50 ml. portion of each of the first three samples and also in the 10 ml. portion of the first sample. On nutrient agar total bacterial counts ranged from less than 1 to 130 per ml., while on T.G.E.M. agar they varied from 140 to 290 per ml. Numbers of proteolytic organisms ranged from 4 to 14 per ml., while those of lipolytic organisms varied from less than 1 to 8 per ml. No Ps. putrefaciens organisms were isolated. In experimental unsalted butter washed with the first, third and fourth samples there was no noticeable flavor deterioration even at 7 days while similar butter washed with the second sample was off in flavor at 4 days.

Plant 7

The three samples of water from the well at plant 7 were satisfactory (table 7). Coliform organisms were absent, total bacterial counts on nutrient agar did not exceed 3 per ml., while on T.G.E.M. agar they ranged from 4 to 20 per ml., proteolytic and lipolytic counts did not exceed 3 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the first sample was off at 4 days, but the defect was not conspicuous enough to be classified even at 7 days. Similar butter washed with the other two samples

Table 7

Results on samples of water from plant 7

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-19-41	-	<1	4	<1	<1	-	O.K. 7 da.
2.	"tank	2-19-41	-	<1	6	<1	2	-	off 6 da.
3.	well	4-16-41	-	<1	6	3	3	-	O.K. 7 da.
4.	tank	4-16-41	-	<1	8	1	1	-	rancid 5 da.
5.	well	12-2-41	-	3	20	<1	<1	-	O.K. 7 da.
6.	tank	12-2-41	/ 50 ml.	4	110	2	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

showed no serious flavor deterioration at 4 days but one sample was cheesy at 5 days.

The first two of three samples of water that had been through a wooden storage tank were satisfactory; the third was unsatisfactory. With the satisfactory samples, coliform organisms were absent, total bacterial counts were less than 1 per ml. on nutrient agar and were 6 and 8 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and no Ps. putrefaciens organisms were isolated. In experimental unsalted butter washed with the samples there was no noticeable flavor deterioration at 4 days. The unsatisfactory sample showed coliform organisms in 50 ml., total bacterial counts were 4 per ml. on nutrient agar and 110 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed no noticeable flavor deterioration at 7 days.

Plant 8

The six samples of well water from plant 8 were satisfactory (table 8). No coliform organisms were detected. On nutrient agar total bacterial counts ranged from less than 1 to 8 per ml., while on T.G.E.M. agar they varied from less than 1 to 50 per ml. Numbers of proteolytic organisms ranged from less than 1 to 22 per ml., those of lipolytic organisms

Table 8

Results on samples of water from plant 8

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	2-5-41	-	3	<1	<1	<1	-	musty 4 da.
2.	"tank	2-5-41	-	2	50	4	<1	-	putrid 4 da.
3.	well	4-9-41	-	1	28	5	1	-	O.K. 7 da.
4.	tank	4-9-41	-	2	1600	225	8	-	cheesy 5 da.
5.	well	1-13-42	-	1	2	2	<1	-	O.K. 7 da.
6.	tank	1-13-42	≠ 50 ml.	250	900	40	50	-	cheesy 7 da.
7.	well	2-24-42	-	<1	15	1	<1	-	O.K. 7 da.
8.	tank	2-24-42	≠ 50 ml.	5	190	10	<1	-	cheesy 6 da.
9.	well	3-18-42	-	6	20	1	<1	-	O.K. 7 da.
10.	tank	3-18-42	-	14	185	27	5	-	cheesy 4 da.
11.	tank	3-18-42	-	8	45	2	<1	-	O.K. 7 da.
12.	well	4-2-42	-	8	50	22	2	-	O.K. 7 da.
13.	tank	4-2-42	-	85	350	5	<1	-	rancid 5 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with one sample displayed a musty flavor at 4 days but no other defects, whereas similar butter washed with the other samples showed no definite flavor deterioration at 7 days.

Seven samples of water that had been through a metal supply tank were examined. The first five samples were unsatisfactory, a sixth sample, following a thorough cleaning of the tank, was satisfactory but a seventh sample, collected about 2 weeks after the cleaning was unsatisfactory. With the unsatisfactory samples, coliform organisms were present in the 50 ml. portion of two samples. Total bacterial counts ranged from 2 to 250 per ml. on nutrient agar and from 50 to 1600 per ml. on T.G.E.M. agar, proteolytic counts varied from 4 to 225 per ml. and lipolytic counts ranged from less than 1 to 50 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with two samples showed serious flavor deterioration at 4 days, while similar butter washed with the other samples showed flavor deterioration at 5, 7, 6 and 5 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was 8 per ml. on nutrient agar and 45 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the sample, there was no discernable flavor deterioration at 7 days.

Plant 9

The three samples of water from the well at plant 9 were unsatisfactory (table 9). Coliform organisms were present in the 50 ml. and 10 ml. portions of each sample and total bacterial counts ranged from less than 1 to 40 per ml. on nutrient agar and from 160 to 200 per ml. on T.G.E.M. agar. Proteolytic counts were 7, 3 and 4 per ml., lipolytic counts were 1, 1 and 14 per ml. and Ps. putrefaciens was not detected. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 4 days but in two instances was rancid at 5 days.

The four samples of water that had been through a metal storage tank were unsatisfactory. Coliform organisms were present in the 50 ml. and 10 ml. portions of each sample and were present in the 1 ml. portion of one sample. Total bacterial counts ranged from less than 1 to 140 per ml. on nutrient agar and from 350 to 900 per ml. on T.G.E.M. agar. Numbers of proteolytic organisms ranged from 1 to 120 per ml. while those of lipolytic organisms varied from less than 1 to 30 per ml. No Ps. putrefaciens organisms were isolated. In experimental unsalted butter washed with the first two samples, there was no noticeable flavor deterioration at 4 days but one lot was cheesy at 5 days. Similar butter washed with the last two samples showed a cheesy flavor in each case at 4 days.

Table 9

Results on samples of water from plant 9

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	"tank	12-11-40	≠ 50,10, 1 ml.	140	900	120	11	-	O.K. 7 da.
2.	well	4-2-41	≠ 50,10 ml.	40	160	7	1	-	O.K. 7 da.
3.	tank	4-2-41	≠ 50,10 ml.	135	500	80	8	-	cheesy 5 da.
4.	well	10-15-41	≠ 50,10 ml.	<1	170	3	1	-	rancid 5 da.
5.	tank	10-15-41	≠ 50,10 ml.	<1	750	1	<1	-	cheesy 4 da.
6.	well	4-14-42	≠ 50,10 ml.	1	200	4	14	-	rancid 5 da.
7.	tank	4-14-42	≠ 50,10 ml.	14	350	30	30	-	cheesy 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

Plant 10

The three samples of well water from plant 10 were satisfactory (table 10). Coliform organisms were absent, on nutrient agar total bacterial counts did not exceed 1 per ml. while on T.G.E.M. agar they varied from 1 to 14 per ml., numbers of proteolytic and lipolytic organisms did not exceed 1 per ml. and Ps. putrefaciens was not recovered. Experimental unsalted butter washed with the samples showed no serious flavor deterioration at 7 days, although in one instance the butter was musty.

Three samples of water that had been through a metal storage tank were examined; one was unsatisfactory and two were satisfactory. With the unsatisfactory sample, no coliform organisms were detected. Total bacterial counts were 13 per ml. on nutrient agar and 1000 per ml. on T.G.E.M. agar, the proteolytic count was 200 per ml., the lipolytic count was 6 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the sample there was no noticeable flavor deterioration at 7 days. The satisfactory samples did not contain coliform organisms, total bacterial counts were each less than 1 per ml. on nutrient agar and were 14 and 3 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with water from the tank showed no noticeable flavor deterioration at 4 days; in one instance the butter was rancid at 6 days while

Table 10

Results on samples of water from plant 10

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	12-4-40	-	1	14	1	1	-	O.K. 7 da.
2.	"tank	12-4-40	-	13	1000	200	6	-	O.K. 7 da.
3.	well	4-23-41	-	<1	2	<1	<1	-	musty 7 da.
4.	tank	4-23-41	-	<1	14	1	2	-	rancid 6 da.
5.	well	1-13-42	-	1	1	<1	<1	-	O.K. 7 da.
6.	tank	1-13-42	-	<1	3	<1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

in the other it showed no defect at 7 days.

Plant 11

The water supply of plant 11 is obtained from the city mains; there are no wells at the plant.

The three samples of city water from the plant were unsatisfactory (table 11). Coliform organisms were absent, total bacterial counts on nutrient agar did not exceed 1 per ml., while on T.G.E.M. agar they ranged from 55 to 250 per ml. Proteolytic counts varied from 3 to 50 per ml. and lipolytic counts ranged from 2 to 30 per ml. Ps. putrefaciens was isolated from the sample showing the lowest total count on T.G.E.M. agar. Experimental unsalted butter washed with the first two samples showed no noticeable flavor deterioration at 4 days, but in one instance the butter was rancid at 6 days. Similar butter washed with the third sample showed rancidity at 4 days.

Plant 12

The two samples of well water from plant 12 were satisfactory (table 12). No coliform organisms were detected. On nutrient agar total bacterial counts were 3 and less than 1 per ml., while on T.G.E.M. agar each count was 20 per ml. Proteolytic counts were 3 and 2 per ml., lipolytic counts were 2 and 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the water showed no

Table 11

Results on samples of water from plant 11

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	city	11-20-40	-	<1	55	3	2	+	O.K. 7 da.
2.	city	3-19-41	-	1	125	4	3	-	rancid 6 da.
3.	city	10-8-41	-	<1	250	50	30	-	rancid 4 da.

*P. = proteolytic
#L. = lipolytic

Table 12

Results on samples of water from plant 12

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	12-11-40	-	3	20	3	2	-	O.K. 7 da.
2.	"tank	12-11-40	-	2	10	2	<1	-	O.K. 7 da.
3.	well	10-15-41	-	<1	20	2	1	-	O.K. 7 da.
4.	tank	10-15-41	-	<1	90	2	1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

noticeable flavor deterioration even at 7 days.

After the water had been through a metal storage tank the two samples again were satisfactory. Coliform organisms were absent, total bacterial counts on nutrient agar were 2 and less than 1 per ml. and on T.G.E.M. agar they were 10 and 90 per ml., each proteolytic count was 2 per ml., the lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. When used to wash experimental unsalted butter the samples produced no noticeable flavor deterioration even at 7 days.

Plant 13

The three samples of water from the well at plant 13 were satisfactory (table 13). Coliform organisms were absent, on nutrient agar total bacterial counts did not exceed 1 per ml., on T.G.E.M. agar they did not exceed 2 per ml., numbers of proteolytic and lipolytic organisms did not exceed 1 per ml. and Ps. putrefaciens was not isolated. When used to wash experimental unsalted butter, the samples did not cause serious deterioration even at 7 days.

Three samples of water that had been through a metal storage tank were examined. The first two were satisfactory while the third was unsatisfactory since coliform organisms were present in the 50 ml., 10 ml. and 1 ml. portions. With the three samples total bacterial counts did not exceed 2 per ml. on nutrient agar and were 4, 4 and 15 per ml., on T.G.E.M.

Table 13

Results on samples of water from plant 13

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	1-22-41	-	<1	1	<1	<1	-	O.K. 7 da.
2.	"tank	1-22-41	-	<1	4	1	1	-	off 6 da.
3.	well	4-16-41	-	<1	2	1	1	-	O.K. 7 da.
4.	tank	4-16-41	-	1	4	2	2	-	rancid 5 da.
5.	well	10-20-41	-	1	2	<1	<1	-	O.K. 7 da.
6.	tank	10-20-41	+ 50,10, 1 ml.	2	15	<1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples displayed no noticeable flavor deterioration at 4 days but in one case the butter was off at 6 days and in one case it was rancid at 5 days.

Plant 14

At plant 14 water from the well commonly is used; occasionally water from the city mains is employed.

The one sample of water from the well was satisfactory (table 14). Coliform organisms were absent, the total bacterial count on nutrient agar was 2 per ml. and on T.G.E.M. agar it was 12 per ml., the proteolytic and lipolytic counts were 2 and 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed no definite flavor deterioration at 4 days but was defective in 5 days.

The three samples of water that had been through a pressure tank were satisfactory. No coliform organisms were detected. On nutrient agar total bacterial counts did not exceed 1 per ml., while on T.G.E.M. agar they were 2, 76 and 13 per ml. Proteolytic counts ranged from 1 to 24 per ml. and lipolytic counts were each less than 1 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the first two samples was satisfactory at 7 days while similar butter washed with the third sample was musty at 3 days.

Table 14

Results on samples of water from plant 14

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar: Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	city	2-26-41	-	<1	125	1	<1	-	O.K. 7 da.
2.	well	2-26-41	-	2	12	2	1	-	putrid 5 da.
3.	"tank	2-26-41	-	<1	2	1	<1	-	O.K. 7 da.
4.	city	5-14-41	-	1	1000	32	<1	-	off in 7 da.
5.	tank	5-14-41	-	2	76	24	<1	-	O.K. 7 da.
6.	city	10-28-41	-	220	850	13	10	-	rancid 5 da.
7.	tank	10-28-41	-	<1	13	1	<1	-	musty 3 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal pressure tank

The three samples of city water from this plant were unsatisfactory. Coliform organisms were absent, total bacterial counts ranged from less than 1 to 220 per ml. on nutrient agar and from 125 to 1000 per ml. on T.G.E.M. agar, proteolytic counts varied from 1 to 32 per ml., lipolytic counts ranged from less than 1 to 10 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the first sample showed no flavor deterioration at 7 days; similar butter washed with the next two samples displayed no noticeable flavor deterioration at 4 days but showed serious deterioration at 7 and 5 days.

Plant 15

The five samples of well water from plant 15 were satisfactory (table 15). Coliform organisms were absent. On nutrient agar total bacterial counts ranged from less than 1 to 3 per ml., while on T.G.E.M. agar they varied from 1 to 33 per ml. Numbers of proteolytic organisms ranged from less than 1 to 8 per ml., while lipolytic counts varied from less than 1 to 2 per ml. Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the samples there was no noticeable flavor deterioration at 7 days.

Five samples of water that had been through a metal storage tank were examined. The first was satisfactory, the next three were unsatisfactory and, the fifth following a thorough cleaning of the tank, was satisfactory. The satis-

Table 15

Results on samples of water from plant 15

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	2-5-41	-	2	1	<1	<1	-	O.K. 7 da.
2.	"tank	2-5-41	-	10	10	1	1	-	O.K. 7 da.
3.	well	5-7-41	-	<1	33	8	2	-	O.K. 7 da.
4.	tank	5-7-41	✓ 50,10 ml.	1	700	120	27	-	rancid 6 da.
5.	well	11-4-41	-	<1	17	<1	<1	-	O.K. 7 da.
6.	tank	11-4-41	✓ 50,10 ml.	175	1000	300	10	-	putrid 3 da.
7.	well	2-24-42	-	3	3	<1	<1	-	O.K. 7 da.
8.	tank	2-24-42	✓ 50 ml.	45	250	70	8	-	off 6 da.
9.	well	3-4-42	-	<1	12	<1	<1	-	O.K. 7 da.
10.	tank	3-4-42	-	1	35	<1	<1	-	O.K. 7 da.

*P. = proteolytic "tank = wooden tank
#L. = lipolytic

factory samples did not contain coliform organisms, total bacterial counts were 10 and 1 per ml. on nutrient agar and 10 and 35 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the samples, there was no observable flavor deterioration at 7 days. With the unsatisfactory samples, coliform organisms were present in the 50 ml. portion of each sample and in the 10 ml. portion of two samples. Total bacterial counts ranged from 1 to 175 per ml. on nutrient agar and from 250 to 1000 per ml. on T.G.E.M. agar. Proteolytic counts varied from 70 to 300 per ml. and lipolytic counts ranged from 8 to 27 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the first and third samples showed no noticeable flavor deterioration at 4 days and serious flavor deterioration at 6 days, while similar butter washed with the other sample displayed serious deterioration at 3 days.

Plant 16

At plant 16 it was not possible to obtain samples directly from the well since there was no outlet between the pump and the tank.

The samples of water from the wooden supply tank were satisfactory on the basis of general bacteriological results, but the first contained Ps. putrefaciens which made it

Table 16

Results on samples of water from plant 16

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	"tank	11-20-40	-	<1	15	3	<1	+	musty 4 da.
2.	tank	2-19-41	-	<1	50	10	1	-	putrid 6 da.
3.	tank	10-8-41	-	1	8	1	<1	-	musty 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

objectionable (table 16). With the three samples coliform organisms were absent, total bacterial counts on nutrient agar did not exceed 1 per ml. and on T.G.E.M. agar they ranged from 8 to 50 per ml., proteolytic counts varied from 1 to 10 per ml. and lipolytic counts did not exceed 1 per ml. Ps. putrefaciens was not isolated from the second and third samples. Experimental unsalted butter washed with two samples displayed a musty flavor at 4 days, while similar butter washed with the other sample was putrid at 6 days.

Plant 17

The first two of three samples of water from the well at plant 17 were satisfactory, while the third was objectionable (table 17) since coliform organisms were present in the 50 ml. portion and also in a second 50 ml. portion studied with a special medium*. With the three samples total bacterial counts were less than 1 per ml. on nutrient agar and did not exceed 3 per ml. on T.G.E.M. agar. Proteolytic and lipolytic counts did not exceed 1 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples displayed no noticeable flavor deterioration at 7 days.

Of three samples of water that had been through a metal storage tank, the first and third were unsatisfactory while the second was satisfactory. With the unsatisfactory samples

* See page 185.

Table 17

Results on samples of water from plant 17

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-19-41	-	<1	<1	<1	<1	-	O.K. 7 da.
2.	"tank	2-19-41	∕ 50 ml.	<1	17	3	2	∕	O.K. 7 da.
3.	well	4-30-41	-	<1	3	<1	1	-	musty 6 da.
4.	tank	4-30-41	-	20	70	<1	2	-	O.K. 7 da.
5.	well	12-2-41	∕ 50 ml.	<1	3	<1	<1	-	O.K. 7 da.
6.	tank	12-2-41	∕ 50 ml.	4	70	5	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

coliform organisms were present in the 50 ml. portion of each. Total bacterial counts on nutrient agar were less than 1 and 4 per ml. and on T.G.E.M. agar they were 17 and 70 per ml. Proteolytic counts were 3 and 5 per ml. and lipolytic counts did not exceed 2 per ml. Ps. putrefaciens was isolated from the first sample. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 7 days. The satisfactory sample did not contain coliform organisms, total bacterial counts were 20 per ml. on nutrient agar and 70 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. No flavor defects were observed at 7 days when this sample was used to wash experimental unsalted butter.

Plant 18

The first three of the four samples of water from the well at plant 18 were unsatisfactory while the fourth was satisfactory (table 18). With the unsatisfactory samples coliform organisms were present in the 50 ml. portions of each and in the 10 ml. portion of one sample, total bacterial counts ranged from 8 to 16 per ml. on nutrient agar and from 17 to 80 per ml. on T.G.E.M. agar, proteolytic counts ranged from 3 to 12 per ml., lipolytic counts varied from less than 1 to 2 per ml. and Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter

Table 18

Results on samples of water from plant 18

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-12-41	+ 50 ml.	12	24	3	2	-	O.K. 7 da.
2.	"tank	2-12-41	+ 50 ml.	140	300	48	6	-	cheesy 4 da.
3.	well	5-7-41	+ 50 ml.	16	80	12	1	-	O.K. 7 da.
4.	tank	5-7-41	+ 50 ml.	5	70	8	1	-	cheesy 4 da.
5.	well	2-24-42	+ 50,10 ml.	8	17	5	<1	-	cheesy 7 da.
6.	tank	2-24-42	+ 50 ml.	29	250	6	1	-	rancid 6 da.
7.	well	4-7-42	-	<1	12	<1	<1	-	O.K. 7 da.
8.	tank	4-7-42	-	1	325	15	9	-	rancid 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

there was no noticeable flavor deterioration at 4 days but in one instance the butter was cheesy at 6 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and 12 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts were each less than 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with this sample showed no noticeable flavor deterioration at 7 days.

The four samples of water that had been through a metal storage tank were unsatisfactory. Coliform organisms were present in the 50 ml. portion of each of three samples. Total bacterial counts ranged from 1 to 140 per ml. on nutrient agar and varied from 70 to 325 per ml. on T.G.E.M. agar. Numbers of proteolytic organisms ranged from 6 to 48 per ml. and those of lipolytic organisms varied from 1 to 9 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with three samples displayed serious flavor deterioration at 4 days, while similar butter washed with one sample showed no noticeable flavor deterioration at 4 days but was rancid at 6 days.

Plant 19

The three samples of water from the well at plant 19 were satisfactory (table 19). No coliform organisms were detected. On nutrient agar total bacterial counts varied from less than 1 to 2 per ml., while on T.G.E.M. agar they ranged from 1 to

Table 19

Results on samples of water from plant 19

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-5-41	-	2	1	<1	<1	-	O.K. 7 da.
2.	"tank	2-5-41	-	160	1700	300	60	-	putrid 4 da.
3.	well	4-16-41	-	<1	2	1	1	-	rancid 5 da.
4.	tank	4-16-41	-	20	325	5	16	-	putrid 4 da.
5.	well	10-20-41	-	1	3	1	1	-	O.K. 7 da.
6.	tank	10-20-41	-	36	750	5	<1	-	musty 6 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

3 per ml. Numbers of proteolytic and lipolytic organisms did not exceed 1 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no definite flavor deterioration at 4 days but one lot was rancid at 5 days and two lots were satisfactory at 7 days.

All of the three samples of water that had been through a metal storage tank were unsatisfactory. Coliform organisms were absent. Total bacterial counts on nutrient agar ranged from 20 to 160 per ml., while on T.G.E.M. agar they varied from 325 to 1700 per ml. Proteolytic counts ranged from 3 to 300 per ml. and lipolytic counts varied from less than 1 to 60 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with two samples was putrid at 4 days while butter washed with one sample displayed no noticeable flavor deterioration at 4 days but was musty at 6 days.

Plant 20

The three samples of well water from plant 20 were satisfactory (table 20). No coliform organisms were detected. On nutrient agar total bacterial counts were each less than 1 per ml. while on T.G.E.M. agar they ranged from 1 to 23 per ml., proteolytic and lipolytic counts did not exceed 3 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with one sample showed a musty flavor at 4 days; butter washed with the other two samples displayed no noticeable flavor deterioration at 7 days.

Table 20

Results on samples of water from plant 20

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	12-4-40	-	<1	12	<1	<1	-	musty 4 da.
2.	"tank	12-4-40	-	<1	10	<1	3	-	O.K. 7 da.
3.	well	4-23-41	-	<1	23	3	3	-	O.K. 7 da.
4.	tank	4-23-41	-	80	200	4	32	-	putrid 4 da.
5.	well	1-13-42	-	<1	1	<1	<1	-	O.K. 7 da.
6.	tank	1-13-42	-	4	27	<1	5	-	sl. rancid 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

Of the three samples of water that had been through a wooden storage tank two were satisfactory while one was unsatisfactory. With the satisfactory samples coliform organisms were absent, total bacterial counts on nutrient agar were less than 1 and 4 per ml. while on T.G.E.M. agar they were 10 and 27 per ml., proteolytic counts were each less than 1 per ml. and lipolytic counts were 3 and 5 per ml. Ps. putrefaciens was not detected. When used to wash experimental unsalted butter, there was no serious flavor deterioration at 7 days although in one instance the butter was slightly rancid. The unsatisfactory sample did not contain coliform organisms, total bacterial counts were 80 per ml. on nutrient agar and 200 per ml. on T.G.E.M. agar, the proteolytic count was 4 per ml., the lipolytic count was 32 per ml. and Ps. putrefaciens was not isolated. When this sample was used to wash experimental unsalted butter, a putrid flavor was evident at 3 days.

Plant 21

All three samples of water from the well at plant 21 were satisfactory (table 21). Coliform organisms were absent; total bacterial counts on nutrient agar did not exceed 2 per ml. while on T.G.E.M. agar they varied from 1 to 4 per ml. Proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no definite flavor

Table 21

Results on samples of water from plant 21

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	11-27-40	-	<1	1	1	<1	-	O.K. 7 da.
2.	well	4-9-41	-	2	4	1	1	-	rancid 5 da.
3.	well	1-20-42	-	<1	2	<1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic

deterioration at 4 days; one sample was rancid at 5 days, while the other two were satisfactory at 7 days.

Plant 22

The four samples of well water from plant 22 were satisfactory (table 22). Coliform organisms were not detected. On nutrient agar total bacterial counts ranged from 1 to 5 per ml., on T.G.E.M. agar they varied from 7 to 40 per ml., proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. None of the experimental unsalted butter washed with the samples was defective at 4 days; three lots were satisfactory at 7 days while one lot was rancid at 5 days.

Four samples of well water that had been through a metal storage tank were examined; the first, second and fourth were satisfactory while the third was unsatisfactory. With the three satisfactory samples no coliform organisms were detected, total bacterial counts did not exceed 2 per ml. on nutrient agar and varied from 4 to 32 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with two samples showed no noticeable flavor deterioration at 4 days (one lot was putrid at 5 days and the other was satisfactory at 7 days), while butter washed with one sample was musty at 3 days. The unsatisfactory sample showed coliform organisms in the 50 ml. and 10 ml.

Table 22

Results on samples of water from plant 22

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-8-41	-	5	40	2	2	-	O.K. 7 da.
2.	"tank	1-8-41	-	2	4	1	1	-	putrid 5 da.
3.	well	3-26-41	-	2	32	1	<1	-	rancid 5 da.
4.	tank	3-26-41	-	<1	32	<1	1	-	musty 3 da.
5.	well	11-11-41	-	1	30	2	1	-	O.K. 7 da.
6.	tank	11-11-41	+ 50,10 ml.	7	750	65	<1	-	unclean 4 da.
7.	well	3-24-41	-	1	7	<1	<1	-	O.K. 7 da.
8.	tank	3-24-41	-	1	15	<1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

portions, total bacterial counts were 7 per ml. on nutrient agar and 750 per ml. on T.G.E.M. agar, the proteolytic count was 65 per ml., the lipolytic count was less than 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with this sample showed definite flavor deterioration at 4 days.

Plant 23

All three samples of water from the well at plant 23 were satisfactory (table 23). Coliform organisms were absent, on nutrient agar total bacterial counts ranged from less than 1 to 2 per ml. while on T.G.E.M. agar they varied from 2 to 24 per ml., proteolytic and lipolytic counts were less than 1 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with two samples there was no noticeable flavor deterioration at 7 days; in butter washed with one sample a musty flavor was evident at 4 days.

The three samples of water that had been through a wooden storage tank were satisfactory on the basis of general bacteriological condition but one sample produced serious deterioration in butter. With the three samples coliform organisms were not detected. On nutrient agar total bacterial counts ranged from less than 1 to 20 per ml. while on T.G.E.M. agar the total counts varied from 4 to 85 per ml. Proteolytic counts ranged from 1 to 4 per ml., lipolytic counts varied from less than 1 to 3 per ml. and Ps. putrefaciens organisms

Table 23

Results on samples of water from plant 23

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	11-13-40	-	<1	2	<1	<1	-	O.K. 7 da.
2.	"tank	11-13-40	-	<1	40	2	3	-	putrid 4 da.
3.	well	3-5-41	-	1	24	<1	<1	-	musty 4 da.
4.	tank	3-5-41	-	<1	4	1	<1	-	O.K. 7 da.
5.	well	11-18-41	-	2	20	<1	<1	-	O.K. 7 da.
6.	tank	11-18-41	-	20	85	4	3	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

were not detected. Experimental unsalted butter washed with one sample showed a putrid condition at 4 days; the total count on T.G.E.M. agar with this sample was 40 per ml. Butter washed with two samples showed no flavor defects at 7 days.

Plant 24

At plant 24 there are two wells. Until March 19, 1941, water from both well A and well B was pumped into a wooden supply tank for use in the plant. However, when the poor quality of the water from well A was evident, its use was discontinued since well B was capable of furnishing sufficient water.

The two samples of water from well A were unsatisfactory (table 24). Coliform organisms were present in the 50 ml. portion of each sample and in the 10 ml. portion of one sample, total bacterial counts were 35 and 800 per ml. on nutrient agar and 120 and 1600 per ml. on T.G.E.M. agar, proteolytic counts were 10 and 300 per ml., lipolytic counts were 6 and 80 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 4 days; with one sample of water the butter was still satisfactory at 7 days, whereas with the other sample the butter was rancid at 6 days.

The two samples of water from well B were questionable in nature. Coliform organisms were absent, on nutrient agar total bacterial counts were 8 and 3 per ml., while on T.G.E.M.

Table 24

Results on samples of water from plant 24

Sample no.	Type sample	Date examined	Test for *Escherichia*-*Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	[a]well	11-20-40	+ 50,10 ml.	35	120	10	6	-	O.K. 7 da.
2.	"tank	11-20-40	+ 50,10 ml.	24	300	20	1	-	O.K. 7 da.
3.	[a]well	3-19-41	+ 50 ml.	800	1600	300	80	-	rancid 6 da.
4.	[b]well	3-19-41	-	8	120	8	1	-	musty 4 da.
5.	tank	3-19-41	+ 50 ml.	200	800	40	25	-	off 7 da.
6.	[b]well	10-8-41	-	3	80	6	4	-	skunk 4 da.
7.	tank	10-8-41	-	13	90	5	2	-	rancid 6 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank
[a]well = well A
[b]well = well B

agar they were 120 and 80 per ml., proteolytic counts were 8 and 6 per ml. and lipolytic counts were 1 and 4 per ml. Ps. putrefaciens was not detected but an organism resembling Pseudomonas mephitica was isolated from one sample. In experimental unsalted butter washed with one sample, a musty flavor was evident at 4 days, while similar butter washed with the other sample (the one yielding Ps. mephitica) had a skunk-like odor at 4 days.

The first two samples that had been through a wooden supply tank represented mixtures of water from two wells; they were unsatisfactory. Coliform organisms were present in the 50 ml. portion of each sample and in the 10 ml. portion of one sample. On nutrient agar total bacterial counts were 24 and 200 per ml., while on T.G.E.M. agar they were 300 and 800 per ml.; proteolytic counts were 20 and 40 per ml. and lipolytic counts were 1 and 25 per ml. Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter, it showed no noticeable flavor deterioration at 4 days; one lot of butter was still satisfactory at 7 days, while the other was off at 7 days.

The one sample of water from well B that had been through the wooden supply tank was satisfactory. Coliform organisms were absent, the total bacterial count on nutrient agar was 13 per ml. and on T.G.E.M. agar it was 90 per ml., there were 5 proteolytic and 2 lipolytic organisms per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter

washed with the sample was satisfactory at 4 days but was rancid at 6 days.

Plant 25

At plant 25 it was not possible to obtain water samples directly from the well since there was no outlet between the pump and the tank.

The four samples of water that had been through the metal storage tank were unsatisfactory (table 25). Coliform organisms were absent, but total bacterial counts on nutrient agar ranged from 16 to 750 per ml. and on T.G.E.M. agar from 240 to 1200 per ml.; proteolytic counts varied from 45 to 300 per ml. and lipolytic counts from 7 to 50 per ml. Ps. putrefaciens was not detected. Experimental unsalted butter washed with one of the samples showed serious flavor deterioration only at 7 days, but with the other three samples deterioration was evident at 4 days in two instances and at 3 days in one instance. The butter showing deterioration only after 7 days was washed with the water sample having the lowest total counts.

Plant 26

The water from the well at plant 26 was unsatisfactory on the first and third examinations and satisfactory on the second and fourth examinations (table 26). With the unsatisfactory samples coliform organisms were present in the 50 ml.

Table 25

Results on samples of water from plant 25

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	"tank	1-15-41	-	16	240	60	7	-	rancid 7 da.
2.	tank	5-7-41	-	24	550	100	8	-	rancid 4 da.
3.	tank	11-11-41	-	750	1200	300	50	-	off 4 da.
4.	tank	4-7-42	-	200	600	45	18	-	cheesy 3 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

Table 26

Results on samples of water from plant 26

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	11-13-40	✓ 50,10 ml.	200	50	2	<1	-	cheesy 4 da.
2.	"tank	11-13-40	✓ 50,10 ml.	1000	240	26	5	-	putrid 4 da.
3.	well	3-5-41	-	<1	6	1	1	-	rancid 5 da.
4.	tank	3-5-41	-	2	16	1	<1	-	musty 5 da.
5.	well	11-25-41	✓ 50,10, 1 ml.	180	650	300	10	-	cheesy-rancid 3 da.
6.	tank	11-25-41	✓ 50,10 ml.	550	1600	300	30	-	off 3 da.
7.	well	4-2-42	-	1	1	<1	<1	-	O.K. 7 da.
8.	tank	4-2-42	-	1	37	1	1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

and 10 ml. portions of each sample and in the 1 ml. portion of one sample. Total bacterial counts were 200 and 180 per ml. on nutrient agar and 50 and 650 per ml. on T.G.E.M. agar, proteolytic counts were 2 and 300 per ml. and lipolytic counts were less than 1 and 10 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples was cheesy at 4 days in one instance and cheesy and rancid at 3 days in the other. The satisfactory samples did not contain coliform organisms, total bacterial counts did not exceed 1 per ml. on nutrient agar and were 6 and 1 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not detected. When experimental unsalted butter was washed with the samples, there was no flavor defects evident at 4 days; one sample was rancid at 5 days while the other was still satisfactory at 7 days.

Four samples of well water that had been through a wooden supply tank were examined. The first and third were unsatisfactory while the second and fourth were satisfactory. With the unsatisfactory samples coliform organisms were present in the 50 ml. and 10 ml. portions of each sample. Total bacterial counts were 1000 and 550 per ml. on nutrient agar and were 240 and 1600 per ml. on T.G.E.M. agar, proteolytic counts were 26 and 300 per ml. and lipolytic counts were 5 and 30 per ml. Ps. putrefaciens was not isolated. The experimental unsalted butter washed with the samples was putrid at 4 days in one instance and off at 3 days in the other. The satis-

factory samples did not contain coliform organisms, total bacterial counts did not exceed 2 per ml. on nutrient agar and were 16 and 37 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter flavor defects were not evident at 4 days; one sample was musty at 5 days while the other was still satisfactory at 7 days.

Plant 27

At plant 27 water from the well commonly is used; occasionally water from the city mains is employed.

The three samples of water from the well were satisfactory (table 27). No coliform organisms were detected. Total bacterial counts on nutrient agar ranged from less than 1 to 22 per ml., while those on T.G.E.M. agar varied from 4 to 18 per ml. Proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no definite flavor deterioration at 7 days.

Three samples of well water that had been through a metal storage tank were examined. The first two were unsatisfactory while the third was satisfactory. With the unsatisfactory samples coliform organisms were present in the 50 ml. portion of each and in the 10 ml. portion of one sample. Total bacterial counts were 13 and 1600 per ml. on nutrient agar and

Table 27

Results on samples of water from plant 27

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-2-41	-	22	6	2	<1	-	O.K. 7 da.
2.	"tank	1-2-41	≠ 50,10 ml.	13	1000	300	40	-	fruity 3 da.
3.	city	1-2-41	-	14	100	5	2	-	O.K. 7 da.
4.	well	4-9-41	-	<1	18	<1	<1	-	O.K. 7 da.
5.	tank	4-9-41	≠ 50 ml.	1600	1800	65	75	-	unclean 3 da.
6.	city	4-9-41	-	<1	200	<1	2	-	off 4 da.
7.	well	1-20-42	-	4	4	1	<1	-	O.K. 7 da.
8.	tank	1-20-42	-	6	85	1	1	-	O.K. 7 da.
9.	city	1-20-42	-	<1	3	<1	<1	-	O.K. 7 da.

*P. = proteolytic "tank = metal tank
#L. = lipolytic

1000 and 1800 per ml. on T.G.E.M. agar, proteolytic counts were 300 and 65 per ml. and lipolytic counts were 40 and 75 per ml. Ps. putrefaciens was not recovered. Experimental unsalted butter washed with the two samples displayed serious flavor deterioration at 3 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was 6 per ml. on nutrient agar and was 85 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. When the sample was used to wash experimental unsalted butter there was no noticeable flavor deterioration at 7 days.

The first and third of three city water samples were satisfactory while the second was unsatisfactory. With the satisfactory samples coliform organisms were absent, total bacterial counts were 14 and less than 1 per ml. on nutrient agar and 100 and 3 per ml. on T.G.E.M. agar, proteolytic counts were 5 and less than 1 per ml., lipolytic counts were 2 and less than 1 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the samples there was no noticeable flavor deterioration at 7 days. The unsatisfactory sample did not contain coliform organisms; the total bacterial count was less than 1 per ml. on nutrient agar but was 200 per ml. on T.G.E.M. agar. Proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed a flavor defect at 4 days.

Plant 28

At plant 28 there are two wells. Water from well A commonly is employed while that from well B is used only in times of shortage.

The first two of the three samples of water from well A were satisfactory but the third was unsatisfactory (table 28). With the satisfactory samples coliform organisms were absent, total bacterial counts did not exceed 3 per ml. on nutrient agar and were 10 and 29 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 4 per ml. and Ps. putrefaciens was not isolated. There was no noticeable flavor deterioration at 7 days when experimental unsalted butter was washed with the samples. The unsatisfactory sample did not contain coliform organisms and the total bacterial count on nutrient agar was less than 1 per ml.; however, the total count on T.G.E.M. agar was 230 per ml. The proteolytic count was 3 per ml., the lipolytic count was 4 per ml. and Ps. putrefaciens was not detected. When experimental unsalted butter was washed with the sample, no serious flavor defect was evident at 7 days.

The first two of the three samples of water from well B were satisfactory; the third was objectionable since coliform organisms were present in the 50 ml. portion and also in a second 50 ml. portion studied with a special medium*. With

* See page 185.

Table 28

Results on samples of water from plant 28

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	[b]well	1-15-41	-	<1	20	1	<1	-	musty 5 da.
2.	[a]well	1-15-41	-	3	10	4	1	-	O.K. 7 da.
3.	[b]well	5-7-41	-	1	20	1	1	-	O.K. 7 da.
4.	[a]well	5-7-41	-	<1	29	1	<1	-	O.K. 7 da.
5.	[b]well	11-11-41	∕ 50 ml.	2	70	1	5	-	O.K. 7 da.
6.	[a]well	11-11-41	-	<1	230	3	4	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
[a]well = well A
[b]well = well B

the three samples total bacterial counts did not exceed 2 per ml. on nutrient agar and were 20, 20 and 70 per ml. on T.G.E.M. agar, proteolytic counts did not exceed 1 per ml. and the lipolytic counts were less than 1, 1 and 5 per ml. Ps. putrefaciens organisms were not isolated. In experimental unsalted butter washed with the samples, there was no flavor deterioration at 4 days; with one sample a musty flavor was evident at 5 days, while with the other samples the butter was satisfactory at 7 days.

Plant 29

The two samples of water from the well at plant 29 were satisfactory (table 29). Coliform organisms were not detected. On nutrient agar total bacterial counts were both less than 1 per ml., while on T.G.E.M. agar they were 5 and 2 per ml. Numbers of proteolytic and lipolytic organisms did not exceed 1 per ml. Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the samples there was no noticeable flavor deterioration at 7 days.

Three samples of water that had been through a wooden storage tank were examined. The first two were unsatisfactory and the third was satisfactory. With the unsatisfactory samples coliform organisms were absent. Total bacterial counts on nutrient agar were less than 1 and 42 per ml. but on T.G.E.M. agar they were 200 and 400 per ml., proteolytic counts were 9 and 40 per ml. and lipolytic counts did not

Table 29

Results on samples of water from plant 29

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	12-4-40	-	<1	5	1	1	-	O.K. 7 da.
2.	"tank	12-4-40	-	<1	200	9	1	-	musty 4 da.
3.	well	4-23-41	-	<1	2	<1	<1	-	O.K. 7 da.
4.	tank	4-23-41	-	42	400	40	2	+	putrid 3 da.
5.	tank	1-13-42	-	<1	38	2	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

exceed 2 per ml. Ps. putrefaciens was isolated from one sample. Experimental unsalted butter washed with one sample had a musty flavor at 4 days, while similar butter washed with the sample yielding Ps. putrefaciens was putrid at 3 days. The satisfactory sample did not show the presence of coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and 38 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens organisms were not found. When the sample was used to wash experimental unsalted butter, no flavor defect was noted at 7 days.

Plant 30

The two samples of water from the well at plant 30 were unsatisfactory (table 30). Coliform organisms were present in the 50 ml. portion of each sample. On nutrient agar total bacterial counts were 20 and 100 per ml., while on T.G.E.M. agar they were 350 and 250 per ml. Proteolytic counts were 30 and 3 per ml. while lipolytic counts were 70 and 40 per ml. Ps. putrefaciens was not isolated. The experimental unsalted butter washed with the samples was rancid at 4 days and 2 days.

The three samples of water that had been through a metal storage tank were unsatisfactory. Coliform organisms were present in the 50 ml. portion of each sample. Total bacterial counts on nutrient agar ranged from 2 to 5 per ml.

Table 30

Results on samples of water from plant 30

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-15-41	∠ 50 ml.	20	350	30	70	-	rancid 4 da.
2.	"tank	1-15-41	∠ 50 ml.	4	280	20	25	-	off 5 da.
3.	well	3-26-41	∠ 50 ml.	100	250	3	40	-	rancid 2 da.
4.	tank	3-26-41	∠ 50 ml.	2	200	10	16	-	putrid 4 da.
5.	tank	11-11-41	∠ 50 ml.	5	550	50	4	-	cheesy 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

and on T.G.E.M. agar from 200 to 550 per ml. Numbers of proteolytic organisms ranged from 10 to 50 per ml. and those of lipolytic organisms from 4 to 25 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with one sample displayed no noticeable flavor deterioration at 4 days but was off at 5 days; butter washed with two samples was putrid at 4 days in one instance and cheesy at 4 days in the other.

Plant 31

The three samples of water from the well at plant 31 were satisfactory (table 31). Coliform organisms were absent. On nutrient agar total bacterial counts ranged from less than 1 to 12 per ml., while on T.G.E.M. agar they varied from 2 to 20 per ml. Proteolytic counts did not exceed 2 per ml. and lipolytic counts did not exceed 3 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no flavor deterioration at 7 days.

The first of three samples of water that had been through a metal storage tank was satisfactory but the others were unsatisfactory. With the satisfactory sample coliform organisms were absent, the total bacterial count was less than 1 per ml. on nutrient agar and 6 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. There were no flavor defects at 7 days when experimental unsalted butter was washed

Table 31

Results on samples of water from plant 31

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	2-12-41	-	12	2	2	<1	-	O.K. 7 da.
2.	"tank	2-12-41	-	<1	6	2	<1	-	O.K. 7 da.
3.	well	5-21-41	-	<1	20	1	3	-	O.K. 7 da.
4.	tank	5-21-41	-	4200	7000	300	200	-	cheesy 3 da.
5.	well	2-24-42	-	<1	12	1	1	-	O.K. 7 da.
6.	tank	2-24-42	-	230	125	5	17	-	rancid 6 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

with the sample. With the unsatisfactory samples coliform organisms were absent, total bacterial counts were 4200 and 230 per ml. on nutrient agar and 7000 and 125 per ml. on T.G.E.M. agar, proteolytic counts were 300 and 5 per ml., lipolytic counts were 200 and 17 per ml. and Ps. putrefaciens was not isolated. When experimental unsalted butter was washed with one sample, a cheesy flavor was evident at 3 days; with the other sample no flavor defect was noticeable at 4 days but the butter was rancid at 6 days.

Plant 32

Both samples of water from the well at plant 32 were satisfactory (table 32). No coliform organisms were detected. On nutrient agar total bacterial counts were less than 1 per ml., while on T.G.E.M. agar they were 2 and 33 per ml. Proteolytic counts were 1 and 3 per ml. and lipolytic counts did not exceed 1 per ml. Ps. putrefaciens was not recovered. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 4 days; one lot was satisfactory at 7 days while the other was rancid at 6 days.

One of the two samples of water that had been through a wooden storage tank was questionable because of a high total count, while the other was satisfactory. The samples showed no coliform organisms. Total bacterial counts on nutrient agar were 4 and less than 1 per ml. and on T.G.E.M. agar they were 120 and 7 per ml.; numbers of proteolytic and lipolytic

Table 32

Results on samples of water from plant 32

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	11-27-40	-	<1	2	1	<1	-	O.K. 7 da.
2.	"tank	11-27-40	-	4	120	<1	1	-	O.K. 7 da.
3.	city	11-27-40	-	1	18	1	<1	-	O.K. 7 da.
4.	city	4-23-41	-	13	125	2	4	+	putrid 4 da.
5.	well	1-13-42	-	<1	33	3	1	-	rancid 6 da.
6.	tank	1-13-42	-	<1	7	1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

organisms did not exceed 1 per ml. and Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter there was no significant flavor deterioration at 7 days.

One of two samples of city water was satisfactory while the other was unsatisfactory. With the satisfactory sample, coliform organisms were absent, the total bacterial counts on nutrient agar was 1 per ml. and on T.G.E.M. agar it was 18 per ml., proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens organisms were not found. When experimental unsalted butter was washed with the sample no flavor deterioration was observed at 7 days. The unsatisfactory sample contained no coliform organisms and the total bacterial count on nutrient agar was 13 per ml.; the total count on T.G.E.M. agar was 125 per ml. The proteolytic count was 2 per ml., and the lipolytic count was 4 per ml. Ps. putrefaciens organisms were isolated and when experimental unsalted butter was washed with the water, a putrid flavor was evident at 4 days.

Plant 33

The first two samples of water from the well at plant 33 were satisfactory, while the third was unsatisfactory (table 33). With the satisfactory samples, coliform organisms were absent, total bacterial counts on nutrient agar did not exceed 2 per ml., while on T.G.E.M. agar they were 1 and 7 per ml.,

Table 33

Results on samples of water from plant 33

Sample no.	Type sample	Date examined	Test for *Escherichia*-*Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-5-41	-	2	1	<1	<1	-	musty 4 da.
2.	"tank	2-5-41	-	40	440	40	6	-	putrid 4 da.
3.	well	5-21-41	-	1	7	1	<1	-	putrid 5 da.
4.	tank	5-21-41	-	<1	80	3	11	-	rancid 4 da.
5.	well	11-4-41	-	<1	325	8	1	-	O.K. 7 da.
6.	tank	11-4-41	-	5	325	5	5	-	rancid 6 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with one sample developed a musty flavor in 4 days; similar butter washed with the other sample showed no flavor deterioration at 4 days but had a rancid flavor at 5 days. The unsatisfactory sample contained no coliform organisms and the total bacterial count was less than 1 per ml. on nutrient agar; however, on T.G.E.M. agar the total count was 325 per ml. The proteolytic count was 8 per ml. and the lipolytic count was 1 per ml. and Ps. putrefaciens organisms were not detected. There was no flavor deterioration at 7 days when the sample was used to wash experimental unsalted butter.

The three samples of water that had been through a metal supply tank were unsatisfactory. Coliform organisms were absent. On nutrient agar total bacterial counts ranged from less than 1 to 40 per ml., while on T.G.E.M. agar they varied from 80 to 440 per ml. Proteolytic counts ranged from 3 to 40 per ml. and lipolytic counts from 5 to 11 per ml. Ps. putrefaciens was not detected. In experimental unsalted butter washed with the first two samples, there was a putrid defect at 4 days in one instance and rancidity at 4 days in the other. Similar butter washed with the third sample showed no noticeable flavor deterioration at 4 days, but was rancid at 6 days.

Plant 34

The three samples of water from the well at plant 34 were satisfactory (table 34). Coliform organisms were absent, total bacterial counts on nutrient agar were 3, less than 1 and less than 1 per ml. and on T.G.E.M. agar they were 1, 3 and 6 per ml., proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the water there was no serious flavor deterioration at 4 days; one lot showed a musty flavor at 6 days, while the other two were still satisfactory at 7 days.

Plant 35

All four of the water samples from the well at plant 35 were satisfactory (table 35). No coliform organisms were detected. On nutrient agar total bacterial counts did not exceed 1 per ml., while on T.G.E.M. agar they varied from 4 to 60 per ml. Proteolytic counts did not exceed 2 per ml. and lipolytic counts ranged from less than 1 to 8 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 4 days; one sample was musty at 5 days, while the others were still satisfactory at 7 days.

Four samples of water that had been through a wooden supply tank were examined. The first two were unsatisfactory,

Table 34

Results on samples of water from plant 34

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	1-22-41	-	3	1	<1	<1	-	musty 6 da.
2.	well	4-16-41	-	<1	3	1	1	-	O.K. 7 da.
3.	well	10-20-41	-	<1	6	1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic

Table 35

Results on samples of water from plant 35

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	2-12-41	-	<1	4	1	1	-	musty 5 da.
2.	"tank	2-12-41	+ 50,10, 1 ml.	600	1000	300	100	-	rancid 2 da.
3.	well	4-16-41	-	<1	32	2	8	-	O.K. 7 da.
4.	tank	4-16-41	+ 50 ml.	180	320	10	11	-	putrid 3 da.
5.	well	11-4-41	-	1	60	1	4	-	O.K. 7 da.
6.	tank	11-4-41	-	2	55	1	1	-	musty 4 da.
7.	well	4-7-42	-	1	7	<1	<1	-	O.K. 7 da.
8.	tank	4-7-42	-	2	5	<1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

while the last two were satisfactory. With the unsatisfactory samples, coliform organisms were present in the 50 ml. portion of each and in the 10 ml. and 1 ml. portions of one sample. Total bacterial counts were 600 and 180 per ml. on nutrient agar and 1000 and 320 per ml. on T.G.E.M. agar, proteolytic counts were 300 and 10 per ml. and lipolytic counts were 100 and 11 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed rancidity at 2 days in one instance and a putrid condition at 3 days in the other. The satisfactory samples did not contain coliform organisms, total bacterial counts did not exceed 2 per ml. on nutrient agar and were 55 and 5 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not detected. A musty flavor was evident at 4 days when one sample was used to wash butter, and no flavor defects were observed at 7 days when the other sample was used.

Plant 36

The five samples of water from the well at plant 36 were unsatisfactory (table 36). Coliform organisms were detected in the 50 and 10 ml. portions of each sample and in the 1 ml. portions of two samples. On nutrient agar total bacterial counts ranged from 5 to 9 per ml., while on T.G.E.M. agar they varied from 220 to 500 per ml. Numbers of proteolytic organisms ranged from 3 to 120 per ml. and those of lipolytic

Table 36

Results on samples of water from plant 36

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-8-41	≠ 50,10 ml.	6	300	20	1	-	O.K. 7 da.
2.	"tank	1-8-41	≠ 50,10, 1 ml.	6	325	40	6	-	musty 7 da.
3.	well	4-2-41	≠ 50,10, 1 ml.	6	500	120	5	-	rancid 2 da.
4.	tank	4-2-41	≠ 50 ml.	80	1300	300	24	-	putrid 3 da.
5.	well	10-15-41	≠ 50,10, 1 ml.	6	220	5	<1	-	rancid 4 da.
6.	tank	10-15-41	≠ 50,10 ml.	100	550	60	40	-	rancid 3 da.
7.	well	3-24-42	≠ 50,10 ml.	5	250	3	1	-	rancid 3 da.
8.	well	3-24-42	≠ 50,10 ml.	9	250	6	4	-	rancid 3 da.
9.	tank	3-24-42	≠ 50,10, 1 ml.	10	400	20	5	-	rancid 3 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

organisms from less than 1 to 5 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the one sample showed no noticeable flavor deterioration at 7 days while butter washed with the other four samples was rancid at 2, 4, 3 and 3 days.

With the unsatisfactory well the four samples of water that had been through a wooden supply tank naturally were unsatisfactory. Coliform organisms were present in the 50 ml. portions of each sample, in the 10 ml. portions of three samples and in the 1 ml. portion of two samples. Total bacterial counts ranged from 6 to 100 per ml. on nutrient agar and from 325 to 1300 per ml. on T.G.E.M. agar. Proteolytic counts ranged from 20 to 300 per ml. and lipolytic counts varied from 5 to 40 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the first sample showed no noticeable flavor deterioration at 4 days but was musty at 7 days. With the other samples the butter was putrid or rancid at 3 days.

Plant 37

All three samples of water from the well at plant 37 were satisfactory, although one sample had a comparatively high count on T.G.E.M. agar, (table 37). Coliform organisms were absent. On nutrient agar total bacterial counts were less than 1 per ml. with two samples and 5 per ml. with one, while on T.G.E.M. agar they varied from 22 to 100 per ml. Neither

Table 37

Results on samples of water from plant 37

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-19-41	-	<1	30	2	4	-	musty 7 da.
2.	"tank	2-19-41	-	<1	40	2	24	-	O.K. 7 da.
3.	well	4-30-41	-	<1	100	4	4	-	O.K. 7 da.
4.	tank	4-30-41	-	16	650	20	20	-	rancid 7 da.
5.	well	12-2-41	-	5	22	<1	<1	-	O.K. 7 da.
6.	tank	12-2-41	-	2	40	20	<1	-	unclean 5 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

the proteolytic or lipolytic counts exceeded 4 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 4 days; one sample was musty at 7 days, while the others were still satisfactory at 7 days.

Three samples of water that had been through a metal supply tank were examined; two were satisfactory while one was unsatisfactory. With the satisfactory samples coliform organisms were absent, total bacterial counts were less than 1 and 2 per ml. on nutrient agar and were each 40 per ml. on T.G.E.M. agar, proteolytic counts were 2 and 20 per ml. and lipolytic counts were 24 and less than 1 per ml. Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter, there was no flavor deterioration at 4 days; one lot had an unclean flavor at 5 days, while the other was still satisfactory at 7 days. The unsatisfactory sample contained no coliform organisms and the total bacterial count on nutrient agar was 16 per ml.; however, the total count on T.G.E.M. agar was 650 per ml. Proteolytic and lipolytic counts were each 20 per ml. Ps. putrefaciens was not isolated. When the sample was used to wash experimental unsalted butter no flavor defect was evident at 4 days, but rancidity was evident at 7 days.

Plant 38

At plant 38 there are two wells. Water from both well

Table 38

Results on samples of water from plant 38

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	"tank	11-20-40	+ 50 ml.	2	1000	25	8	-	off 4 da.
2.	tank	11-20-40	-	8	1000	100	13	-	off 6 da.
3.	[b]well	3-19-41	-	100	1200	300	200	-	cheesy 3 da.
4.	[a]well	3-19-41	-	2	100	3	<1	-	O.K. 7 da.
5.	tank	3-19-41	-	50	1500	300	50	-	cheesy 3 da.
6.	[b]well	10-8-41	+ 50,10 ml.	5	400	10	8	+	rancid 7 da.
7.	[a]well	10-8-41	-	2	16	2	2	-	O.K. 7 da.
8.	tank	10-8-41	+ 50,10 ml.	55	800	80	32	+	sl. rancid 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank
[a]well = well A
[b]well = well B

A and well B is pumped into a wooden supply tank for use in the plant.

The two samples of water from well A were satisfactory although one had a relatively high count on T.G.E.M. agar (table 38). Coliform organisms were absent, on nutrient agar total bacterial counts were 2 per ml. in each case while on T.G.E.M. agar they were 100 and 16 per ml., proteolytic counts were 3 and 2 per ml. and lipolytic counts were less than 1 and 2 per ml. Ps. putrefaciens was not isolated. In experimental unsalted butter washed with these samples there was no definite flavor deterioration at 7 days.

The two samples of water from well B were unsatisfactory. Coliform organisms were present in the 50 ml. and 10 ml. portions of one sample. Total bacterial counts were 100 and 5 per ml. on nutrient agar and 1200 and 400 per ml. on T.G.E.M. agar., proteolytic counts were 300 and 10 per ml. and lipolytic counts were 200 and 8 per ml. Ps. putrefaciens was isolated from the one sample. Experimental unsalted butter washed with one sample was cheesy at 3 days, while butter washed with the other sample showed no noticeable flavor deterioration at 4 days but was rancid at 7 days.

The four samples representing mixtures of water from the two wells that had been through a wooden supply tank were unsatisfactory. Coliform organisms were present in the 50 ml. portions of two samples and in the 10 ml. portion of one of these. Total bacterial counts on nutrient agar ranged from 2

to 55 per ml., while on T.G.E.M. agar they varied from 800 to 1500 per ml. Proteolytic counts ranged from 25 to 300 per ml. and lipolytic counts from 8 to 50 per ml. Ps. putrefaciens was isolated from one sample. Experimental unsalted butter washed with two samples displayed definite flavor deterioration at 4 and 3 days; similar butter washed with the other samples showed no noticeable flavor deterioration at 4 days, but one lot was off at 6 days and the other was slightly rancid at 7 days.

Plant 39

All three samples of water from the well at plant 39 were satisfactory (table 39). No coliform organisms were detected. Total bacterial counts on nutrient agar were less than 1, less than 1 and 8 per ml. while on T.G.E.M. agar they were 12, 12 and 40 per ml. Proteolytic counts ranged from less than 1 to 7 per ml., lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no serious flavor deterioration at 4 days; however, one lot was musty at 3 days, one was musty at 5 days and one was putrid at 5 days.

Plant 40

The four samples of water from the well at plant 40 were satisfactory (table 40). Coliform organisms were absent, total bacterial counts did not exceed 2 per ml. on nutrient

Table 39

Results on samples of water from plant 39

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	1-8-41	-	<1	12	7	<1	-	musty 5 da.
2.	well	4-2-41	-	<1	12	<1	<1	-	musty 3 da.
3.	well	10-15-41	-	8	40	2	2	-	putrid 5 da.

*P. = proteolytic
#L. = lipolytic

Table 40

Results on samples of water from plant 40

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-26-41	-	2	4	<1	<1	-	O.K. 7 da.
2.	"tank	2-26-41	-	1	8	5	1	-	putrid 4 da.
3.	well	5-14-41	-	<1	34	1	1	-	rancid 7 da.
4.	tank	5-14-41	-	<1	65	3	<1	-	cheesy 7 da.
5.	well	10-28-41	-	<1	19	2	1	-	O.K. 7 da.
6.	tank	10-28-41	/ 50 ml.	7	220	9	<1	-	cheesy 3 da.
7.	well	4-21-42	-	2	28	1	<1	-	O.K. 7 da.
8.	tank	4-21-42	-	1100	1500	100	<1	-	unclean 3 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

agar while on T.G.E.M. agar they varied from 4 to 34 per ml., proteolytic organisms did not exceed 2 per ml., lipolytic organisms did not exceed 1 per ml. and Ps. putrefaciens was not detected. Experimental unsalted butter washed with the samples displayed no flavor deterioration at 4 days; one sample was rancid at 7 days, while the others were still satisfactory at 7 days.

Four samples of well water that had been through a wooden supply tank were examined. Two samples were satisfactory on the basis of general bacteriological information, although when butter was washed with one of the samples a putrid defect was evident at 4 days. The other two samples were unsatisfactory. With the two satisfactory samples coliform organisms were not detected, total bacterial counts did not exceed 1 per ml. on nutrient agar and were 8 and 65 per ml. on T.G.E.M. agar, proteolytic counts were 5 and 3 per ml. and lipolytic counts did not exceed 1 per ml. Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter a putrid flavor was evident at 4 days in one case; no flavor defect was evident at 4 days in the other but the butter was cheesy at 7 days. The unsatisfactory samples showed coliform organisms in the 50 ml. portion of one sample, total bacterial counts on nutrient agar were 7 and 1100 per ml. and on T.G.E.M. agar they were 220 and 1500 per ml., proteolytic counts were 9 and 100 per ml. and lipolytic counts were less than 1 per ml. in each case.

Ps. putrefaciens was not found. When the water was used to wash experimental unsalted butter, a cheesy flavor was present at 3 days in one lot and an unclean flavor at 3 days in the other.

Plant 41

Four of five samples of well water from plant 41 were unsatisfactory, while one was satisfactory (table 41). With the unsatisfactory samples coliform organisms were found in the 50 ml. portion of three and in the 10 ml. portion of one. Total bacterial counts on nutrient agar varied from 2 to 3 per ml. while on T.G.E.M. agar they ranged from 11 to 325 per ml. Proteolytic counts varied from 2 to 5 per ml. and lipolytic counts from less than 1 to 4 per ml. Ps. putrefaciens was isolated from one sample. When experimental unsalted butter was washed with the samples, three lots were putrid or cheesy at 4 days while the other lot was still satisfactory at 7 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was 1 per ml. on nutrient agar and 40 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not detected. When experimental unsalted butter was washed with the sample it was satisfactory at 7 days.

Of the five samples of water that had been through a metal supply tank, four were unsatisfactory and one was satis-

Table 41

Results on samples of water from plant 41

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-15-41	-	2	11	2	<1	≠	putrid 4 da.
2.	"tank	1-15-41	-	30	200	5	30	-	musty 5 da.
3.	well	3-26-41	-	1	40	1	<1	-	O.K. 7 da.
4.	tank	3-26-41	-	3	60	1	3	-	O.K. 7 da.
5.	well	11-11-41	≠ 50,10 ml.	3	325	5	3	-	O.K. 7 da..
6.	tank	11-11-41	≠ 50 ml.	3	200	20	3	-	unclean 7 da.
7.	well	4-14-42	≠ 50 ml.	3	80	3	<1	-	cheesy 4 da.
8.	tank	4-14-42	-	16	160	<1	10	-	cheesy 5 da.
9.	well	4-14-42	≠ 50 ml.	2	110	4	4	-	cheesy 4 da.
10.	tank	4-14-42	-	11	200	1	7	-	rancid 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

factory. With the unsatisfactory samples coliform organisms were present in the 50 ml. portion of one sample. Total bacterial counts ranged from 3 to 30 per ml. on nutrient agar and from 160 to 200 per ml. on T.G.E.M. agar, proteolytic counts varied from less than 1 to 20 per ml. and lipolytic counts ranged from 3 to 30 per ml. Ps. putrefaciens was not recovered. When experimental unsalted butter was washed with the samples, three lots were unclean, cheesy or rancid at 4 days; one lot showed no defect at 4 days but was musty at 5 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was 3 per ml. on nutrient agar and 60 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts did not exceed 3 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed no defect at 7 days.

Plant 42

At plant 42 there are two wells. Water from well A is most frequently used while that from well B is used only in emergencies.

All four samples of water from well A were unsatisfactory (table 42). Coliform organisms were present in the 50 ml. portion of each sample and in the 10 ml. portions of two samples. On nutrient agar total bacterial counts ranged from less than 1 to 90 per ml., while on T.G.E.M. agar they varied from 35 to 650 per ml. Numbers of proteolytic organisms

Table 42

Results on samples of water from plant 42

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	[a]well	12-11-40	≠ 50,10 ml.	5	65	9	20	-	rancid 3 da.
2.	[a]well	4-2-41	≠ 50,10 ml.	90	650	30	12	-	putrid 2 da.
3.	[b]well	4-2-41	≠ 50 ml.	30	325	25	8	-	cheesy 3 da.
4.	[a]well	10-15-41	≠ 50 ml.	<1	46	2	1	-	sl. rancid 7 da.
5.	[a]well	4-14-42	≠ 50 ml.	2	35	2	<1	-	O.K. 7 da.
6.	[b]well	4-14-42	-	60	300	20	40	-	fruity 4 da.

*P. = proteolytic
#L. = lipolytic
[a]well = well A
[b]well = well B

ranged from 2 to 30 per ml. while those of lipolytic organisms varied from less than 1 to 20 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with two samples was rancid at 3 days in one case and putrid at 2 days in the other; butter washed with the remaining samples showed no serious flavor deterioration at 7 days, although one lot showed slight rancidity at 7 days.

The two samples of water from well B were unsatisfactory. Coliform organisms were present in the 50 ml. portion of one sample, the total bacterial counts on nutrient agar were 30 and 60 per ml. and on T.G.E.M. agar were 325 and 300 per ml., proteolytic counts were 25 and 20 per ml., lipolytic counts were 8 and 40 per ml. and Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter, one lot was cheesy at 3 days and one was fruity at 4 days.

Plant 43

All three samples of water from the well at plant 43 were satisfactory (table 43). No coliform organisms were detected. On nutrient agar total bacterial counts did not exceed 1 per ml., while on T.G.E.M. agar they varied from 8 to 50 per ml. Proteolytic counts ranged from less than 1 to 5 per ml. and lipolytic counts from less than 1 to 3 per ml. Ps. putrefaciens was not recovered. Experimental unsalted butter washed with one sample was rancid at 4 days; similar butter

Table 43

Results on samples of water from plant 43

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	12-11-40	-	<1	16	1	2	-	O.K. 7 da.
2.	"tank	12-11-40	-	1	30	5	1	-	O.K. 7 da.
3.	well	4-2-41	-	<1	8	<1	<1	-	O.K. 7 da.
4.	[a]tank	4-2-41	-	2	24	1	1	-	cheesy 4 da.
5.	well	11-18-41	-	1	50	5	3	-	rancid 4 da.
6.	[a]tank	11-18-41	∕ 50,10 ml.	<1	35	3	3	-	rancid 7 da.
7.	[a]tank	3-24-42	-	1	11	<1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank
[a]tank = glass-lined tank

washed with the remaining samples was still satisfactory at 7 days.

The one sample of well water that had been through a metal supply tank was satisfactory. Coliform organisms were absent, the total bacterial count on nutrient agar was 1 per ml. and on T.G.E.M. agar it was 30 per ml., proteolytic and lipolytic counts did not exceed 5 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with this sample there was no flavor deterioration at 7 days.

Two of the three samples of water that had been through a glass-lined supply tank were satisfactory on the basis of general bacteriological condition, but when one sample was used to wash experimental unsalted butter there was serious flavor deterioration at 4 days for no evident reason; the remaining sample was unsatisfactory. With the satisfactory samples, coliform organisms were absent, total bacterial counts did not exceed 2 per ml. on nutrient agar and were 24 and 11 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not recovered. Experimental unsalted butter washed with one sample showed a cheesy condition at 4 days; the total count on T.G.E.M. agar with this sample was only 24 per ml. and fluorescent organisms were not noted. Butter washed with the other sample was still satisfactory at 7 days. The unsatisfactory sample contained coliform organisms in the 50 ml. and 10 ml. portions, total bacterial counts were less than 1 per

ml. on nutrient agar and 35 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts were each 3 per ml. and Ps. putrefaciens was not isolated. Butter washed with the sample was satisfactory at 4 days but was rancid at 7 days.

Plant 44

One of the two samples of water from the well at plant 44 was satisfactory while the other was unsatisfactory (table 44). With the satisfactory sample coliform organisms were absent, on nutrient agar the total bacterial count was less than 1 per ml. while on T.G.E.M. agar it was 8 per ml., the proteolytic count was less than 1 per ml. and the lipolytic count was 7 per ml. Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the sample there was no flavor deterioration at 7 days. The unsatisfactory sample did not contain coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar but was 1000 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not found. Experimental unsalted butter washed with the sample was satisfactory at 4 days but had an unclean flavor at 5 days.

Of the three samples of water that had been through a metal pressure tank, one was satisfactory and two were unsatisfactory. The satisfactory sample did not contain coliform organisms, the total bacterial count on nutrient agar was less than 1 per ml. and on T.G.E.M. agar it was 40 per

Table 44

Results on samples of water from plant 44

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-5-41	-	<1	8	<1	7	-	O.K. 7 da.
2.	"tank	2-5-41	-	<1	40	1	7	-	musty 7 da.
3.	tank	5-7-41	-	1	450	5	10	-	O.K. 7 da.
4.	well	10-15-41	-	<1	1000	<1	1	-	unclean 5 da.
5.	tank	10-15-41	-	<1	800	40	10	-	unclean 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal pressure tank

ml., the proteolytic count was 1 per ml. and the lipolytic count was 7 per ml. Ps. putrefaciens was not recovered. Experimental unsalted butter washed with the sample showed no flavor defect at 4 days, while a musty flavor was evident at 7 days. With the unsatisfactory samples coliform organisms were absent. Total bacterial counts did not exceed 1 per ml. on nutrient agar but were 450 and 800 per ml. on T.G.E.M. agar; proteolytic counts were 5 and 40 per ml., lipolytic counts were each 10 per ml. and Ps. putrefaciens organisms were not recovered. When experimental unsalted butter was washed with the samples it showed serious flavor deterioration at 4 days.

Plant 45

Of the three samples of water from the well at plant 45 one was unsatisfactory while two were satisfactory (table 45). With the unsatisfactory sample coliform organisms were not detected, the total bacterial count was 2 per ml. on nutrient agar and 200 per ml. on T.G.E.M. agar, the proteolytic count was 100 per ml., the lipolytic count was 2 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed a cheesy flavor at 4 days. With the satisfactory samples coliform organisms were not recovered, total bacterial counts did not exceed 1 per ml. on nutrient agar and were 3 and 14 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 3 per ml. and

Table 45

Results on samples of water from plant 45

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-2-41	-	2	200	100	2	-	cheesy 4 da.
2.	well	5-14-41	-	<1	3	1	<1	-	O.K. 7 da.
3.	well	10-28-41	-	1	14	3	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic

Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the two samples was still satisfactory at 7 days.

Plant 46

At plant 46 water from the well commonly is used, although water from the city mains occasionally is required.

The three samples of water from the well were unsatisfactory (table 46). Coliform organisms were present in the 50 ml. and 10 ml. portions of one sample, total bacterial counts ranged from less than 1 to 120 per ml. on nutrient agar and from 150 to 375 per ml. on T.G.E.M. agar, proteolytic counts varied from 22 to 125 per ml. and lipolytic counts ranged from 3 to 65 per ml. Ps. putrefaciens was isolated from the sample yielding coliform organisms. Experimental unsalted butter washed with one sample was rancid at 2 days. Butter washed with the other two samples showed no noticeable flavor deterioration at 4 days; one of the samples was still satisfactory at 7 days but the other was off at 7 days.

One of the four samples of city water collected at the plant was satisfactory, but the other three were unsatisfactory. The satisfactory sample did not contain coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and was 80 per ml. on T.G.E.M. agar, the proteolytic count was 24 per ml. and the lipolytic count was 1 per ml. Ps. putrefaciens was not detected. The experi-

Table 46

Results on samples of water from plant 46

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-26-41	-	<1	150	50	32	-	rancid 2 da.
2.	city	2-26-41	-	<1	80	24	1	-	rancid 6 da.
3.	city	2-26-41	-	2	1000	75	24	-	rancid 7 da.
4.	well	5-14-41	-	3	220	22	3	-	O.K. 7 da.
5.	city	5-14-41	∕ 50 ml.	1	260	39	3	∕	rancid 7 da.
6.	city	10-28-41	∕ 50,10 ml.	28	210	60	15	∕	rancid 7 da.
7.	well	10-28-41	∕ 50,10 ml.	120	375	125	65	∕	off 7 da.

*P. = proteolytic
#L. = lipolytic

mental unsalted butter washed with the sample showed no flavor defect at 4 days, but a rancid condition was evident at 6 days. With the unsatisfactory samples coliform organisms were present in the 50 ml. portions of two samples and in the 10 ml. portion of one sample. On nutrient agar total bacterial counts ranged from 1 to 28 per ml., while on T.G.E.M. agar they varied from 210 to 1000 per ml. Proteolytic counts ranged from 39 to 75 per ml. and lipolytic counts from 3 to 24 per ml. Ps. putrefaciens was isolated from the two samples yielding coliform organisms. Experimental unsalted butter washed with the samples showed no serious flavor defect at 4 days but each of the samples was rancid at 7 days.

Plant 47

All three samples of water from the well at plant 47 were satisfactory (table 47). Coliform organisms were absent. On nutrient agar total bacterial counts ranged from 1 to 3 per ml., while on T.G.E.M. agar they varied from 1 to 14 per ml. Proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the samples no noticeable flavor deterioration was observed at 4 days; one lot had a musty flavor at 7 days while the others still were satisfactory.

Of the three samples of water that had been through a metal supply tank, two were unsatisfactory while one was satisfactory. With the unsatisfactory samples coliform

Table 47

Results on samples of water from plant 47

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-8-41	-	1	2	<1	1	-	O.K. 7 da.
2.	"tank	1-8-41	-	30	475	6	3	-	musty 5 da.
3.	well	3-26-41	-	2	1	<1	<1	-	musty 7 da.
4.	tank	3-26-41	-	1	20	2	4	-	musty 4 da.
5.	well	11-18-41	-	3	14	<1	<1	-	O.K. 7 da.
6.	tank	11-18-41	-	70	650	5	1	-	rancid 6 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

organisms were absent. Total bacterial counts were 30 and 70 per ml. on nutrient agar and 475 and 650 per ml. on T.G.E.M. agar, proteolytic counts were 6 and 5 per ml., lipolytic counts were 3 and 1 per ml. and Ps. putrefaciens was not detected. Experimental unsalted butter washed with the samples showed no flavor defects at 4 days; one lot was musty at 5 days, while the other was rancid at 6 days. The satisfactory sample did not contain coliform organisms, total bacterial counts were 1 and 20 per ml. on nutrient agar and T.G.E.M. agar, respectively, the proteolytic count was 2 per ml., the lipolytic count was 4 per ml. and Ps. putrefaciens was not recovered. When the sample was used to wash experimental unsalted butter there was a musty flavor at 4 days.

Plant 48

Of the four samples of water from the well at plant 48, two were unsatisfactory; the other two were satisfactory on the basis of general bacteriological condition but when one sample was used to wash experimental unsalted butter a rancid flavor was evident at 4 days (table 48). With the unsatisfactory samples coliform organisms were present in the 50 ml. portion of one sample (also in a second 50 ml. portion used in a special medium*). Total bacterial counts were each 1 per ml. on nutrient agar and 120 and 27 per ml. on T.G.E.M. agar, proteolytic counts were 30 and 1 per ml. and lipolytic counts

* See page 185.

Table 48

Results on samples of water from plant 48

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-12-41	-	1	120	30	15	-	rancid 2 da.
2.	"tank	2-12-41	-	<1	100	24	3	-	cheesy 4 da.
3.	well	5-21-41	-	<1	31	1	4	-	rancid 4 da.
4.	tank	5-21-41	-	<1	32	2	<1	-	O.K. 7 da.
5.	well	10-20-41	∕ 50 ml.	1	27	1	<1	-	rancid 7 da.
6.	tank	10-20-41	-	140	275	100	1	-	musty 4 da.
7.	well	4-7-42	-	<1	2	1	<1	-	O.K. 7 da.
8.	tank	4-7-42	-	200	390	<1	120	-	rancid 2 da.
9.	city	4-7-42	-	<1	230	9	<1	-	O.K. 7 da.

*P. = proteolytic "tank = metal tank
#L. = lipolytic

were 15 and less than 1 per ml. Ps. putrefaciens was not detected. Experimental unsalted butter washed with the one sample was rancid at 2 days while similar butter washed with the other sample was satisfactory at 4 days but rancid at 7 days. The satisfactory samples contained no coliform organisms, total bacterial counts were each less than 1 per ml. on nutrient agar and were 31 and 2 per ml. on T.G.E.M. agar, proteolytic counts did not exceed 1 per ml. and the lipolytic counts were 4 and less than 1 per ml. Ps. putrefaciens was not found. When the samples were used to wash experimental unsalted butter one lot was rancid at 4 days while the other showed no defect at 7 days.

Four samples of water that had been through a metal supply tank were examined; three were unsatisfactory while one was satisfactory. With the unsatisfactory samples coliform organisms were absent. On nutrient agar total bacterial counts ranged from less than 1 to 200 per ml., while on T.G.E.M. agar they varied from 100 to 390 per ml. Proteolytic counts ranged from less than 1 to 100 per ml. and lipolytic counts from 1 to 120 per ml. Ps. putrefaciens was not recovered. Experimental unsalted butter washed with one sample was rancid at 2 days, butter washed with one sample was cheesy at 4 days and butter washed with one sample was musty at 4 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and 32 per ml. on T.G.E.M. agar, proteolytic

and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. No flavor defect was evident at 7 days when the sample was used to wash experimental unsalted butter.

The one city water sample from this plant was unsatisfactory. Coliform organisms were absent and the total bacterial count on nutrient agar was less than 1 per ml.; however, the count on T.G.E.M. agar was 230 per ml. The proteolytic count was 9 per ml., the lipolytic count was less than 1 per ml. and Ps. putrefaciens was not found. When the sample was used to wash experimental unsalted butter rancidity was evident at 2 days.

Plant 49

All four samples of water from the well at plant 49 were unsatisfactory (table 49). Coliform organisms were present in the 50 ml. and 10 ml. portions of each sample and in the 1 ml. portions of three samples. On nutrient agar total bacterial counts ranged from 6 to 140 per ml. while on T.G.E.M. agar they varied from 15 to 1200 per ml. Numbers of proteolytic organisms ranged from 2 to 100 per ml. and numbers of lipolytic organisms from less than 1 to 75 per ml. Ps. putrefaciens was isolated from two samples. Experimental unsalted butter washed with one sample developed a musty flavor in 4 days while similar butter washed with the other samples showed serious flavor deterioration at 4, 3 and 4

Table 49

Results on samples of water from plant 49

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	12-11-40	≠ 50,10 ml.	6	15	2	3	-	musty 4 da.
2.	"tank	12-11-40	≠ 50,10, 1 ml.	20	700	4	5	≠	putrid 4 da.
3.	well	3-26-41	≠ 50,10, 1 ml.	80	1200	100	40	≠	putrid 4 da.
4.	tank	3-26-41	≠ 50,10, 1 ml.	170	1000	100	80	≠	putrid 2 da.
5.	well	11-18-41	≠ 50,10, 1 ml.	140	700	5	<1	≠	off 3 da.
6.	tank	11-18-41	≠ 50,10, 1 ml.	150	550	6	<1	≠	off 3 da.
7.	well	3-24-42	≠ 50,10, 1 ml.	100	1000	100	75	-	unclean 4 da.
8.	tank	3-24-42	≠ 50,10, 1 ml.	125	1400	100	100	-	cheesy 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

days.

The four samples of well water that had been through a metal supply tank were unsatisfactory. Coliform organisms were present in the 50 ml., 10 ml. and 1 ml. portions of each sample. On nutrient agar total bacterial counts ranged from 20 to 170 per ml. while on T.G.E.M. agar they varied from 550 to 1400 per ml. Proteolytic counts ranged from 4 to 100 per ml. and lipolytic counts from less than 1 to 100 per ml. Ps. putrefaciens was isolated from three samples. In experimental unsalted butter washed with the samples there was serious, but somewhat variable, flavor deterioration at 4, 2, 3, and 4 days.

Plant 50

The water supply of plant 50 is obtained from the city mains, there being no wells at the plant.

The five samples of city water from the plant were unsatisfactory (table 50); one was unsatisfactory primarily because the butter washed with it was rancid in 4 days and the T.G.E.M. agar plates showed fluorescent organisms. Coliform organisms were present in the 50 ml. portions of three samples. On nutrient agar total bacterial counts ranged from less than 1 to 5 per ml. while on T.G.E.M. agar they varied from 12 to 150 per ml. Proteolytic counts ranged from 1 to 6 per ml. and lipolytic counts from 1 to 3 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed

Table 50

Results on samples of water from plant 50

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	"tank	11-13-40	≠ 50,10, 1 ml.	1100	10000	300	45	-	cheesy 4 da.
2.	city	11-13-40	-	<1	150	6	3	-	putrid 6 da.
3.	city	3-5-41	-	5	28	1	1	-	rancid 4 da.
4.	city	11-18-41	≠ 50 ml.	2	90	3	2	-	rancid 6 da.
5.	city	4-14-42	≠ 50 ml.	1	26	5	3	-	O.K. 7 da.
6.	city	4-14-42	≠ 50 ml.	3	12	5	1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

with four samples showed no significant flavor defects at 4 days; two lots had off-flavors at 6 days. Similar butter washed with one sample that contained fluorescent bacteria was rancid at 4 days.

One sample of city water that had been through a storage tank was examined and found unsatisfactory. Coliform organisms were present in the 50 ml., 10 ml. and 1 ml. portions. Total bacterial counts on nutrient agar and T.G.E.M. agar were 1100 and 10,000 per ml., respectively, and there were 300 proteolytic bacteria and 45 lipolytic bacteria per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with this water showed a limburger flavor at 4 days.

Plant 51

All five samples of water from the well at plant 51 were unsatisfactory (table 51). Coliform organisms were present in the 50 ml. portion of each sample and in the 10 ml. portions of two samples. On nutrient agar total bacterial counts ranged from 2 to 10 per ml. while on T.G.E.M. agar they varied from 42 to 1000 per ml. Numbers of proteolytic organisms ranged from 5 to 600 per ml. and those of lipolytic organisms from 1 to 65 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with four samples showed no significant flavor deterioration at 4 days; two of the lots had defects at 5 days, one at 7 days and one was still satisfactory at 7 days. Butter washed with the other

Table 51

Results on samples of water from plant 51

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-29-41	≠ 50,10 ml.	2	80	5	2	-	O.K. 7 da.
2.	"tank	1-29-41	≠ 50,10 ml.	20	700	200	100	-	cheesy 4 da.
3.	well	3-5-41	≠ 50 ml.	10	1000	300	65	-	putrid 3 da.
4.	tank	3-5-41	≠ 50,10 ml.	4	1000	85	10	-	putrid 3 da.
5.	well	5-27-41	≠ 50 ml.	5	80	12	5	-	rancid 7 da.
6.	tank	5-27-41	≠ 50,10 ml.	1	110	15	7	-	off 7 da.
7.	well	11-25-41	≠ 50 ml.	2	42	5	1	-	rancid 5 da.
8.	tank	11-25-41	≠ 50 ml.	7	400	24	<1	-	cheesy 5 da.
9.	well	4-2-42	≠ 50,10 ml.	2	700	600	1	-	unclean 5 da.
10.	tank	4-2-42	≠ 50,10, 1 ml.	300	1200	100	100	-	rancid 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

sample was putrid at 3 days.

The five samples of water that had been through a wooden storage tank were unsatisfactory. Coliform organisms were present in the 50 ml. portion of each sample, in the 10 ml. portions of four samples and in the 1 ml. portion of one sample. On nutrient agar total bacterial counts ranged from 1 to 300 per ml. while on T.G.E.M. agar they varied from 110 to 1200 per ml. Numbers of proteolytic organisms ranged from 15 to 200 per ml. and those of lipolytic organisms from less than 1 to 100 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with three samples had serious flavor defects at 4, 3 and 4 days. Butter washed with the other two samples had no flavor defects at 4 days; one was off in flavor at 7 days and the other was cheesy at 5 days.

Plant 52

Two of the six samples of water from the well at plant 52 were satisfactory, while the other four were unsatisfactory (table 52). The satisfactory samples did not contain coliform organisms, total bacterial counts did not exceed 2 per ml. on nutrient agar and were 2 and 32 per ml. on T.G.E.M. agar, proteolytic counts were less than 1 and 25 per ml., lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter no flavor defects were evident at 7 days. With the unsatisfactory samples coliform organisms were

Table 52

Results on samples of water from plant 52

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-29-41	-	<1	2	<1	<1	-	O.K. 7 da.
2.	well	1-29-41	-	2	32	25	1	-	O.K. 7 da.
3.	well	5-27-41	-	<1	130	24	5	-	rancid 7 da.
4.	well	11-25-41	≠ 50,10 ml.	27	270	10	6	-	musty 3 da.
5.	well	4-2-42	≠ 50 ml.	2	90	5	<1	-	O.K. 7 da.
6.	well	4-2-42	≠ 50,10 ml.	3	24	4	2	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic

present in the 50 ml. portion of each of three samples and in the 10 ml. portions of two samples. Total bacterial counts ranged from less than 1 to 27 per ml. on nutrient agar and from 24 to 270 per ml. on T.G.E.M. agar. Proteolytic counts varied from 4 to 24 per ml. and lipolytic counts from less than 1 to 6 per ml. Ps. putrefaciens was not found. Experimental unsalted butter washed with one sample was musty at 3 days. Butter washed with the other samples showed no flavor defects at 4 days; one lot was rancid at 7 days, while the others were still satisfactory at 7 days.

Plant 53

Two of the three samples of water from the well at plant 53 were satisfactory, while one was unsatisfactory because of the presence of coliform organisms (table 53). With the satisfactory samples coliform organisms were not detected, total bacterial counts on nutrient agar did not exceed 2 per ml., while on T.G.E.M. agar they were 48 and 35 per ml., proteolytic counts were 2 and 8 per ml., lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 4 days; one lot was cheesy at 7 days. The unsatisfactory sample showed coliform organisms in the 50 ml. and 10 ml. portions, the total bacterial count was 3 per ml. on nutrient agar and 55 per ml. on T.G.E.M. agar, the proteolytic count was 2 per ml., the

Table 53

Results on samples of water from plant 53

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-26-41	-	1	48	2	1	-	O.K. 7 da.
2.	"tank	2-26-41	+ 50 ml.	<1	150	32	1	-	putrid 4 da.
3.	well	5-14-41	-	2	35	8	<1	-	cheesy 7 da.
4.	tank	5-14-41	-	24	85	6	1	-	rancid 6 da.
5.	well	10-8-41	+ 50,10 ml.	3	55	2	4	-	O.K. 7 da.
6.	tank	10-8-41	+ 50 ml.	3	700	150	150	-	rancid 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

lipolytic count was 4 per ml. and Ps. putrefaciens was not found. In experimental unsalted butter washed with the sample no significant flavor defects were observed at 7 days.

Two of the three samples of water that had been through a wooden supply tank were unsatisfactory and one was satisfactory. With the unsatisfactory samples coliform organisms were present in the 50 ml. portion of each. Total bacterial counts did not exceed 3 per ml. on nutrient agar and were 150 and 700 per ml. on T.G.E.M. agar, proteolytic counts were 32 and 150 per ml. and lipolytic counts were 1 and 150 per ml. Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter a putrid condition was evident at 4 days in one case and rancidity at 4 days in the other. The satisfactory sample showed no coliform organisms, the total bacterial count was 24 per ml. on nutrient agar and 85 per ml. on T.G.E.M. agar, the proteolytic count was 6 per ml., the lipolytic count was 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed no flavor defect at 4 days but was rancid at 6 days.

Plant 54

Two of three samples of water from the well at plant 54 were satisfactory, while one was unsatisfactory (table 54). With the satisfactory samples coliform organisms were absent, total bacterial counts were less than 1 and 1 per ml. on

Table 54

Results on samples of water from plant 54

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-12-41	-	1	6	3	2	-	musty 5 da.
2.	well	5-21-41	-	<1	28	3	<1	-	off 7 da.
3.	well	11-4-41	+ 50,10 ml.	6	700	100	<1	+	unclean 7 da.

*P. = proteolytic
#L. = lipolytic

nutrient agar and 6 and 28 per ml. on T.G.E.M. agar, proteolytic counts were 3 and 3 per ml., lipolytic counts were 2 and less than 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with two samples showed no noticeable flavor deterioration at 4 days; one lot was musty at 5 days while the other was off at 7 days. The unsatisfactory sample showed coliform organisms in the 50 ml. and 10 ml. portions, total bacterial counts were 6 and 700 per ml. on nutrient and T.G.E.M. agar, respectively, the proteolytic count was 100 per ml., the lipolytic count was less than 1 per ml. and Ps. putrefaciens was isolated. Experimental unsalted butter washed with the sample showed no noticeable flavor deterioration at 4 days but had an unclean flavor at 7 days.

Plant 55

Both samples of water from the well at plant 55 were unsatisfactory, one primarily because it produced rancidity in the experimental butter and contained fluorescent organisms (table 55). Coliform organisms were absent from one sample but present in the 50 ml. and 10 ml. portions of the other. On nutrient agar total bacterial counts were 6 and 3 per ml. while on T.G.E.M. agar they were 40 and 140 per ml., proteolytic counts were 5 and 42 per ml., lipolytic counts were 3 and 18 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples was

Table 55

Results on samples of water from plant 55

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-29-41	-	6	40	5	3	-	rancid 4 da.
2.	"tank	1-29-41	-	150	150	3	20	-	putrid 4 da.
3.	well	5-27-41	+ 50,10 ml.	3	140	42	18	-	rancid 4 da.
4.	tank	5-27-41	+ 50,10 ml.	9	200	55	12	-	off 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

rancid at 4 days in each case.

The two samples of water that had been through a metal storage tank were unsatisfactory. Coliform organisms were absent from one sample and present in the 50 ml. and 10 ml. portions of the other. Total bacterial counts on nutrient agar were 150 and 9 per ml. while on T.G.E.M. agar they were 150 and 200 per ml., proteolytic counts were 3 and 55 per ml. and lipolytic counts were 20 and 12 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the samples was putrid or off at 4 days.

T.G.E.M. agar plates poured with each of the four samples showed fluorescent colonies.

Plant 56

Three of the four samples of water from the well at plant 56 were satisfactory, while one was unsatisfactory (table 56). With the satisfactory samples coliform organisms were absent. Total bacterial counts were each less than 1 per ml. on nutrient agar and ranged from 2 to 6 per ml. on T.G.E.M. agar. Proteolytic and lipolytic counts did not exceed 1 per ml. Ps. putrefaciens was not detected. Experimental unsalted butter washed with one sample displayed a musty flavor at 4 days, while butter washed with the other samples showed no flavor defects at 7 days. The unsatisfactory sample contained no coliform organisms, the total bacterial count on nutrient agar was less than 1 per ml. and on T.G.E.M. agar it was 230

Table 56

Results on samples of water from plant 56

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	"tank	1-8-41	-	80	200	20	40	-	off 4 da.
2.	well	4-2-41	-	<1	2	1	<1	-	musty 4 da.
3.	well	10-15-41	-	<1	230	4	1	-	O.K. 7 da.
4.	tank	10-15-41	-	<1	650	150	10	-	O.K. 7 da.
5.	well	2-3-42	-	<1	2	<1	<1	-	O.K. 7 da.
6.	well	3-24-42	-	<1	6	<1	<1	-	O.K. 7 da.
7.	tank	3-24-42	-	<1	8	<1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

per ml., the proteolytic and lipolytic counts did not exceed 4 per ml. and Ps. putrefaciens was not found. In experimental unsalted butter washed with the sample there was no significant flavor deterioration at 7 days.

Of the three samples of water that had been through a metal supply tank two were unsatisfactory and one was satisfactory. With the unsatisfactory samples coliform organisms were absent, the total bacterial counts on nutrient agar were 80 and less than 1 per ml. and on T.G.E.M. agar they were 200 and 650 per ml., proteolytic counts were 20 and 150 per ml. and lipolytic counts were 40 and 10 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with one sample was off in flavor at 4 days; butter washed with the other sample was still satisfactory at 7 days. The satisfactory sample did not show coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and was 8 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts were each less than 1 per ml. and Ps. putrefaciens was not isolated. When the sample was used to wash experimental unsalted butter there was no flavor defect at 7 days.

Plant 57

At plant 57 water from the well commonly is used, although water from the city mains occasionally is required.

The one sample of city water from the plant was unsatisfactory (table 57). Coliform organisms were present in the

Table 57

Results on samples of water from plant 57

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	city	1-29-41	+ 50 ml.	240	90	10	10	-	putrid 2 da.
2.	well	5-27-41	-	1	100	5	5	-	O.K. 7 da.
3.	tank	5-27-41	-	<1	50	<1	<1	-	O.K. 7 da.
4.	well	11-25-41	-	1	110	2	<1	-	O.K. 7 da.
5.	tank	11-25-41	-	90	330	6	9	-	cheesy-rancid 3 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

50 ml. portion, the total bacterial count on nutrient agar was 240 per ml. and on T.G.E.M. agar it was 90 per ml., there were 10 proteolytic and 10 lipolytic organisms per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed a putrid condition at 2 days.

The two samples of water from the well were questionable because of the relatively high total bacterial counts on T.G.E.M. agar. Coliform organisms were absent. On nutrient agar total counts did not exceed 1 per ml., but on T.G.E.M. agar they were 100 and 110 per ml. Proteolytic counts were 5 and 2 per ml. and lipolytic counts were 5 and less than 1 per ml. Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the samples there was no significant flavor deterioration at 7 days.

Of the two samples of water that had been through a metal supply tank, one was satisfactory and the other was unsatisfactory. With the satisfactory sample coliform organisms were absent, the total bacterial count was 1 per ml. on nutrient agar and 50 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts were each less than 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample was not defective at 7 days. The unsatisfactory sample did not contain coliform organisms, the total count was 90 per ml. on nutrient agar and 330 per ml. on T.G.E.M. agar, the proteolytic count was 6 per ml., the lipolytic count was 9 per ml. and Ps. putrefaciens was not

detected. Experimental unsalted butter washed with this sample was cheesy and rancid at 3 days.

Plant 58

Two of the four samples of water from the well at plant 58 were satisfactory, while the other two were unsatisfactory (table 58). The satisfactory samples did not contain coliform organisms, total bacterial counts did not exceed 2 per ml. on nutrient agar and were 9 and 34 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not recovered. When the samples were used to wash experimental unsalted butter, one lot was musty at 4 days while the other was still satisfactory at 7 days. With the unsatisfactory samples coliform organisms were absent. Total bacterial counts were 3 and 900 per ml. on nutrient agar and 260 and 500 per ml. on T.G.E.M. agar, proteolytic counts were 20 and 8 per ml. and lipolytic counts were 10 and 8 per ml. Ps. putrefaciens was not found. In experimental unsalted butter washed with the samples, a musty flavor was evident at 3 days in one instance while there was no flavor defect at 7 days in the other.

Plant 59

One of the three samples of water from the well at plant 59 was unsatisfactory and two were satisfactory (table 59). The unsatisfactory sample showed coliform organisms in the 50

Table 58

Results on samples of water from plant 58

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	11-27-40	-	1	9	1	2	-	musty 4 da.
2.	well	5-27-41	-	3	260	20	10	-	O.K. 7 da.
3.	well	11-25-41	-	900	500	8	8	-	musty 3 da.
4.	well	4-2-42	-	2	34	1	1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic

Table 59

Results on samples of water from plant 59

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°
1.	well	11-13-40	+ 50,10, 1 ml.	2	80	8	5	-	limburger 4 da.
2.	well	3-5-41	-	5	28	3	<1	-	rancid 5 da.
3.	well	1-20-42	-	1	80	12	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic

ml., 10 ml. and 1 ml. portions, total bacterial counts were 2 per ml. on nutrient agar and 80 per ml. on T.G.E.M. agar, the proteolytic count was 8 per ml., the lipolytic count was 5 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample showed a limburger flavor at 4 days. With the two satisfactory samples coliform organisms were absent, total bacterial counts were 5 and 1 per ml. on nutrient agar and 28 and 80 per ml. on T.G.E.M. agar, proteolytic counts were 3 and 12 per ml., lipolytic counts were each less than 1 per ml. and Ps. putrefaciens was not isolated. When the samples were used to wash experimental unsalted butter there was no noticeable flavor deterioration at 4 days; one lot was rancid at 5 days and the other was still satisfactory at 7 days.

Plant 60

At plant 60 it was not possible to obtain samples directly from the well because of the lack of an outlet between the pump and tank.

The three samples of water that had been through the metal storage tank were unsatisfactory (table 60). Coliform organisms were not detected in one sample but were present in the 50 ml. portions of two samples and in the 10 ml. portion of one of these. Total bacterial counts on nutrient agar ranged from 1 to 100 per ml. and on T.G.E.M. agar from 400 to 1000 per ml., proteolytic counts varied from 5 to 300 per ml.

Table 60

Results on samples of water from plant 60

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	"tank	11-20-40	-	20	400	5	11	-	musty 4 da.
2.	tank	3-19-41	+ 50,10 ml.	100	1000	300	65	-	rancid 3 da.
3.	tank	10-20-41	+ 50 ml.	1	800	40	18	-	rancid 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

while lipolytic counts ranged from 11 to 65 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with one sample was musty at 4 days, that washed with one sample was rancid at 3 days while that washed with one sample showed no noticeable flavor deterioration at 4 days but was rancid at 7 days.

Plant 61

One of the two samples of water from the well at plant 61 was unsatisfactory, while the second was satisfactory (table 61). The unsatisfactory sample did not contain coliform organisms. The total bacterial count was less than 1 per ml. on nutrient agar but on T.G.E.M. agar it was 1000 per ml. The proteolytic count was 160 per ml., the lipolytic count was 24 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the sample was cheesy at 2 days. In the satisfactory sample coliform organisms were not found, the total bacterial count was less than 1 per ml. on nutrient agar and 48 per ml. on T.G.E.M. agar, proteolytic and lipolytic counts did not exceed 3 per ml. and Ps. putrefaciens was not detected. When the sample was used to was experimental unsalted butter, no significant flavor defects were evident at 7 days.

The one sample of well water that had been through a concrete supply tank was unsatisfactory. Coliform organisms were absent and the total bacterial count on nutrient agar was

Table 61

Results on samples of water from plant 61

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	2-12-41	-	<1	1000	160	24	-	cheesy 2 da.
2.	well	5-7-41	-	<1	48	3	2	-	O.K. 7 da.
3.	"tank	2-24-42	-	2	400	34	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = concrete tank

2 per ml. but on T.G.E.M. agar the total count was 400 per ml. There were 34 proteolytic and less than 1 lipolytic organisms per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the sample there was no flavor deterioration at 7 days.

Plant 62

Two of the three samples of water from the well at plant 62 were unsatisfactory, while one was satisfactory (table 62). With the unsatisfactory samples coliform organisms were present in the 50 ml. and 10 ml. portions of one. On nutrient agar total bacterial counts were 100 and 12 per ml., while on T.G.E.M. agar they were 120 and 200 per ml. Proteolytic counts were 4 and 5 per ml. and lipolytic counts 33 and 3 per ml. Ps. putrefaciens was not recovered. Experimental unsalted butter washed with one sample had a cheesy flavor at 3 days; that washed with the other sample showed no flavor deterioration at 4 days but was rancid at 7 days. The satisfactory sample showed no coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and 25 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts were each less than 1 per ml. and Ps. putrefaciens was not isolated. When experimental unsalted butter was washed with the sample there was no significant flavor defect at 7 days.

Three of the four samples of water that had been through a wooden supply tank were unsatisfactory, while one was satis-

Table 62

Results on samples of water from plant 62

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	"tank	1-22-41	≠ 50 ml.	3	4200	300	10	≠	putrid 2 da.
2.	tank	3-19-41	≠ 50 ml.	22	300	25	<1	-	cheesy 6 da.
3.	well	5-21-41	-	100	120	4	33	-	rancid 7 da.
4.	well	10-20-41	-	<1	25	<1	<1	-	O.K. 7 da.
5.	tank	10-20-41	-	<1	41	1	<1	-	O.K. 7 da.
6.	well	4-7-42	≠ 50,10 ml.	12	200	5	3	-	cheesy 3 da.
7.	tank	4-7-42	≠ 50,10 ml.	<1	15	<1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

factory. With the unsatisfactory samples coliform organisms were present in the 50 ml. portion of each and in the 10 ml. portion of one. Total bacterial counts ranged from less than 1 to 22 per ml. on nutrient agar and from 15 to 4200 per ml. on T.G.E.M. agar. Proteolytic counts varied from less than 1 to 300 per ml. and lipolytic counts from less than 1 to 10 per ml. Ps. putrefaciens was isolated from one sample. Experimental unsalted butter washed with the sample yielding Ps. putrefaciens was putrid at 2 days; butter washed with the other samples showed no flavor deterioration at 4 days, but one lot was cheesy at 6 days, while the other was still satisfactory at 7 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and 41 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not recovered. When the sample was used to wash experimental unsalted butter no significant flavor defect was observed at 7 days.

Plant 63

Of the four samples of water from the well at plant 63, three were unsatisfactory and one was satisfactory (table 63). With the unsatisfactory samples coliform organisms were present in the 50 ml. and 10 ml. portions of two. Total bacterial counts ranged from 1 to 35 per ml. on nutrient agar and from 7 to 200 per ml. on T.G.E.M. agar. Proteolytic

Table 63

Results on samples of water from plant 63

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	11-13-40	+ 50,10 ml.	35	50	6	<1	-	cheesy 4 da.
2.	"tank	11-13-40	+ 50,10 ml.	2	80	8	5	-	cheesy 4 da.
3.	well	3-5-41	-	2	200	2	1	-	putrid 5 da.
4.	tank	3-5-41	+ 50 ml.	2	150	7	4	-	putrid 3 da.
5.	city	3-5-41	-	2	225	80	<1	-	rancid 3 da.
6.	well	1-20-42	-	<1	46	<1	<1	-	O.K. 7 da.
7.	tank	1-20-42	-	<1	40	2	<1	-	O.K. 7 da.
8.	well	4-2-42	+ 50,10 ml.	1	7	1	<1	-	O.K. 7 da.
9.	tank	4-2-42	+ 50,10 ml.	2	12	1	1	-	O.K. 7 da.

*P. = proteolytic "tank = metal tank
#L. = lipolytic

counts varied from 1 to 6 per ml. and lipolytic counts did not exceed 1 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with one sample showed a cheesy flavor at 4 days; butter washed with the others displayed no noticeable flavor deterioration at 4 days, but one lot was putrid at 5 days, while the other was still satisfactory at 7 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and 46 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts were each less than 1 per ml. and Ps. putrefaciens was not recovered. When the sample was used to wash experimental unsalted butter no significant flavor defect was observed even at 7 days.

Four samples of water that had been through a metal supply tank were examined; three were unsatisfactory, while one was satisfactory. With the unsatisfactory samples coliform organisms were present in the 50 ml. portion of each, in the 10 ml. portions of two of these. Total bacterial counts on nutrient agar did not exceed 2 per ml., while on T.G.E.M. agar they ranged from 12 to 150 per ml. Proteolytic counts varied from 1 to 8 per ml. and lipolytic counts from 1 to 5 per ml. Ps. putrefaciens was not detected. When experimental unsalted butter was washed with the samples one lot was cheesy at 4 days, one was putrid at 3 days, while one was still satisfactory at 7 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was

less than 1 per ml. on nutrient agar and 40 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not found. When the sample was used to wash experimental unsalted butter there was no serious flavor deterioration at 7 days.

The one sample of city water from this plant was unsatisfactory. Coliform organisms were absent, the total bacterial count on nutrient agar was 2 per ml. and on T.G.E.M. agar it was 225 per ml., there were 80 proteolytic and less than 1 lipolytic organisms per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the sample a rancid condition was evident at 3 days.

Plant 64

The four samples of water from the well at plant 64 were satisfactory (table 64). Coliform organisms were absent. On nutrient agar total bacterial counts ranged from less than 1 to 19 per ml., while on T.G.E.M. agar they varied from 3 to 60 per ml. Proteolytic counts varied from less than 1 to 6 per ml. and lipolytic counts from less than 1 to 3 per ml. Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the samples there was no noticeable flavor deterioration at 4 days; one lot was rancid at 6 days while the others showed no defect at 7 days.

Table 64

Results on samples of water from plant 64

Sample no.	Type sample	Date examined	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for Ps. putrefaciens	Action in experimental unsalted butter at 21°C.
1.	well	12-4-40	-	<1	15	4	3	-	O.K. 7 da.
2.	"tank	12-4-40	-	15	1000	100	12	-	O.K. 7 da.
3.	well	4-30-41	-	<1	3	<1	2	-	rancid 6 da.
4.	tank	4-30-41	+ 50,10 ml.	200	1000	200	50	+	putrid 2 da.
5.	well	12-2-41	-	19	12	<1	1	-	O.K. 7 da.
6.	tank	12-2-41	+ 50,10 ml.	70	650	10	<1	+	unclean 6 da.
7.	well	4-21-42	-	4	60	6	<1	-	O.K. 7 da.
8.	tank	4-21-42	+ 50,10 ml.	800	1800	45	70	-	cheesy 2 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

The four samples of well water that had been through a wooden supply tank were unsatisfactory. Coliform organisms were present in the 50 ml. and 10 ml. portions of three samples. Total bacterial counts on nutrient agar ranged from 15 to 800 per ml. while on T.G.E.M. agar they varied from 650 to 1800 per ml. Numbers of proteolytic organisms ranged from 10 to 200 per ml. and those of lipolytic organisms varied from less than 1 to 70 per ml. Ps. putrefaciens was isolated from two of the samples. Experimental unsalted butter washed with the two samples was putrid or cheesy at 2 days. Butter washed with the other samples showed no defect at 4 days; one lot was unclean at 6 days and the other was still satisfactory at 7 days.

Plant 65

All three samples of water from the well at plant 65 were satisfactory (table 65). Coliform organisms were absent. Total bacterial counts on nutrient agar were each less than 1 per ml. and on T.G.E.M. agar they varied from 22 to 70 per ml. Numbers of proteolytic organisms ranged from 5 to 20 per ml. and those of lipolytic organisms from 1 to 8 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the two samples showed no significant flavor deterioration at 7 days, while similar butter washed with one sample was musty at 4 days.

Two of the three samples of water that had been through a

Table 65

Results on samples of water from plant 65

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-5-41	-	<1	22	6	1	-	O.K. 7 da.
2.	"tank	2-5-41	-	<1	28	10	<1	-	putrid 4 da.
3.	well	5-7-41	-	<1	50	5	8	-	O.K. 7 da.
4.	tank	5-7-41	-	<1	300	45	5	-	rancid 5 da.
5.	well	11-4-41	-	<1	70	20	6	-	musty 4 da.
6.	tank	11-4-41	-	<1	65	25	4	-	cheesy 3 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

metal supply tank were satisfactory on the basis of general bacteriological information, but butter washed with the samples developed serious flavor defects at 4 and 3 days. The other samples was unsatisfactory. With the two satisfactory samples coliform organisms were not detected, total bacterial counts were each less than 1 per ml. on nutrient agar and were 28 and 65 per ml. on T.G.E.M. agar, proteolytic counts were 10 and 25 per ml. and lipolytic counts did not exceed 4 per ml. Ps. putrefaciens was not found. When the samples were used to wash butter a putrid flavor was evident at 4 days in one case and a cheesy flavor was evident at 3 days in the other. The unsatisfactory sample did not contain coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and 300 per ml. on T.G.E.M. agar, the proteolytic count was 45 per ml., the lipolytic count was 5 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the sample no flavor defect was noticeable at 4 days; a rancid flavor was evident at 5 days.

Plant 66

All four samples of water from the well at plant 66 were unsatisfactory (table 66). Coliform organisms were present in the 50 ml. portion of one sample. On nutrient agar total bacterial counts did not exceed 1 per ml. while on T.G.E.M. agar they varied from 90 to 500 per ml. Proteolytic counts

Table 66

Results on samples of water from plant 66

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-12-41	-	<1	500	60	8	-	O.K. 7 da.
2.	"tank	2-12-41	+ 50,10 ml.	150	1600	75	70	-	cheesy 2 da.
3.	well	5-21-41	-	<1	200	9	1	-	cheesy 4 da.
4.	tank	5-21-41	+ 50 ml.	3	1900	90	14	-	cheesy-rancid 4 da.
5.	well	11-4-41	-	<1	475	11	<1	-	musty 7 da.
6.	tank	11-4-41	-	1	550	8	2	-	rancid 7 da.
7.	well	4-7-42	+ 50 ml.	1	90	19	<1	-	unclean 5 da.
8.	tank	4-7-42	+ 50 ml.	2	110	12	<1	-	unclean 5 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

ranged from 9 to 60 per ml. and lipolytic counts from less than 1 to 8 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with three samples showed no noticeable flavor deterioration at 4 days; one lot was unclean at 5 days, one lot was musty at 7 days while the third lot showed no defect at 7 days. Similar butter washed with the fourth sample was cheesy at 4 days.

The four samples of water that had been through a metal supply tank also were unsatisfactory. Coliform organisms were present in the 50 ml. portions of three samples and in the 10 ml. portion of one of these. Total bacterial counts on nutrient agar ranged from 1 to 150 per ml. while on T.G.E.M. agar they varied from 110 to 1900 per ml. Proteolytic counts ranged from 8 to 90 per ml. and lipolytic counts from less than 1 to 70 per ml. Ps. putrefaciens was not isolated. Experimental unsalted butter washed with the two samples was cheesy at 2 days in one case and cheesy and rancid at 4 days in the other. Butter washed with the other samples showed no noticeable flavor deterioration at 4 days but one lot was rancid at 7 days and the other had an unclean flavor at 5 days.

Plant 67

One of the three samples of water from the well at plant 67 was satisfactory, the other two being unsatisfactory (table 67). The satisfactory sample did not contain coliform

Table 67

Results on samples of water from plant 67

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	11-20-40	-	2	12	2	1	-	O.K. 7 da.
2.	"tank	11-20-40	≠ 50 ml.	200	1100	350	20	-	off 4 da.
3.	well	3-19-41	≠ 50 ml.	4	90	8	15	-	putrid 6 da.
4.	tank	3-19-41	≠ 50,10 ml.	5	200	30	25	-	rancid 7 da.
5.	well	10-8-41	≠ 50 ml.	<1	24	2	<1	-	O.K. 7 da.
6.	tank	10-8-41	-	7	55	3	10	-	rancid 5 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

organisms, the total bacterial count was 2 per ml. on nutrient agar and 12 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not isolated. There was no flavor deterioration evident at 7 days when the sample was used to wash experimental unsalted butter. With the unsatisfactory samples coliform organisms were present in the 50 ml. portion of each. Total bacterial counts on nutrient agar did not exceed 4 per ml. while on T.G.E.M. agar they were 90 and 24 per ml., proteolytic counts were 8 and 2 per ml. and lipolytic counts 15 and less than 1 per ml. Ps. putrefaciens was not found. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 4 days; one lot was putrid at 6 days, while the other was still satisfactory at 7 days.

Two of the three samples of water that had been through a wooden supply tank were unsatisfactory, while one was satisfactory. With the unsatisfactory samples coliform organisms were present in the 50 ml. portion of two and in the 10 ml. portion of one of these. On nutrient agar total bacterial counts were 200 and 5 per ml. and on T.G.E.M. agar they were 1100 and 200 per ml., proteolytic counts were 350 and 30 per ml. and lipolytic counts 20 and 25 per ml. Ps. putrefaciens was not detected. In experimental unsalted butter washed with the samples one lot was off at 4 days; the other showed no defect at 4 days but was rancid at 7 days. The satisfactory

sample did not contain coliform organisms, the total bacterial count was 7 per ml. on nutrient agar and 55 per ml. on T.G.E.M. agar, the proteolytic count was 3 per ml., the lipolytic count was 10 per ml. and Ps. putrefaciens was not isolated. When experimental unsalted butter was washed with the sample there was no observable flavor deterioration at 4 days but a rancid flavor was evident at 5 days.

Plant 68

At plant 68 water from the well commonly is used, although water from the city mains occasionally is required.

All three samples of water from the well at plant 68 were satisfactory (table 68). Coliform organisms were absent, on nutrient agar total bacterial counts did not exceed 2 per ml., while on T.G.E.M. agar they ranged from 4 to 90 per ml., proteolytic counts varied from 2 to 30 per ml., lipolytic counts each were less than 1 per ml. and Ps. putrefaciens was not isolated. In experimental unsalted butter washed with the samples there was no significant flavor defect at 7 days.

Three of the four samples of water that had been through a concrete supply tank were unsatisfactory while the remaining sample was satisfactory. With the unsatisfactory samples coliform organisms were absent. Total bacterial counts on nutrient agar did not exceed 3 per ml., while on T.G.E.M. agar they ranged from 125 to 160 per ml. Proteolytic counts were 100, 80 and 40 per ml. and lipolytic counts did not exceed 1

Table 68

Results on samples of water from plant 68

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-2-41	-	1	20	18	<1	-	O.K. 7 da.
2.	"tank	1-2-41	-	2	150	100	1	-	musty 6 da.
3.	well	5-14-41	-	<1	4	2	<1	-	O.K. 7 da.
4.	tank	5-14-41	-	<1	33	6	<1	-	cheesy 6 da.
5.	city	10-28-41	-	95	550	70	14	-	cheesy 3 da.
6.	well	4-21-42	-	2	90	30	<1	-	O.K. 7 da.
7.	tank	4-21-42	-	3	160	80	<1	-	unclean 4 da.
8.	tank	4-21-42	-	<1	125	40	<1	-	unclean 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = concrete tank

per ml. Ps. putrefaciens was not detected. Experimental unsalted butter washed with one sample displayed no serious flavor defects at 4 days but was musty at 6 days; butter washed with the other samples had an unclean flavor at 4 days. The satisfactory sample showed no coliform organisms, the total bacterial count was less than 1 per ml. on nutrient agar and 33 per ml. on T.G.E.M. agar, the proteolytic count was 6 per ml., the lipolytic count was less than 1 per ml. and Ps. putrefaciens was not found. When the sample was used to wash experimental unsalted butter there was no serious defect at 4 days but a cheesy flavor was evident at 6 days.

The one sample of city water from this plant was unsatisfactory. Coliform organisms were absent, the total bacterial count on nutrient agar was 95 per ml. and on T.G.E.M. agar it was 550 per ml., there were 70 proteolytic and 14 lipolytic organisms per ml. and Ps. putrefaciens was not recovered. In experimental unsalted butter washed with the sample a cheesy flavor was evident at 3 days.

Plant 69

Two of the three samples of water from the well at plant 69 were satisfactory, while one was unsatisfactory (table 69). With the satisfactory samples coliform organisms were absent. Total bacterial counts were each less than 1 per ml. on nutrient agar and were 7 and 3 per ml. on T.G.E.M. agar, proteolytic counts did not exceed 1 per ml. and lipolytic

Table 69

Results on samples of water from plant 69

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	2-19-41	-	<1	7	1	2	-	O.K. 7 da.
2.	"tank	2-19-41	-	70	600	24	30	-	putrid 6 da.
3.	well	4-30-41	-	<1	240	25	2	-	O.K. 7 da.
4.	tank	4-30-41	+ 50 ml.	16	130	16	3	+	putrid 7 da.
5.	well	12-2-41	-	<1	3	1	<1	-	O.K. 7 da.
6.	tank	12-2-41	-	2	45	1	<1	-	O.K. 7 da.

*P. = proteolytic
#L. = lipolytic
"tank = metal tank

counts did not exceed 2 per ml. Ps. putrefaciens was not recovered. Experimental unsalted butter washed with the samples showed no noticeable flavor deterioration at 7 days. The unsatisfactory sample did not contain coliform organisms. The total bacterial count was less than 1 per ml. on nutrient agar but was 240 per ml. on T.G.E.M. agar. The proteolytic count was 25 per ml., the lipolytic count was 2 per ml. and Ps. putrefaciens was not detected. When the sample was used to wash experimental unsalted butter there was no serious flavor defect at 7 days.

Two of the three samples of water that had been through a metal storage tank were unsatisfactory; one was satisfactory. With the unsatisfactory samples coliform organisms were present in the 50 ml. portion of one sample. Total bacterial counts were 70 and 16 per ml. on nutrient agar and 600 and 130 per ml. on T.G.E.M. agar, proteolytic counts were 24 and 16 per ml. and lipolytic counts were 30 and 3 per ml. Ps. putrefaciens was isolated from one sample. In experimental unsalted butter washed with the samples there was no flavor deterioration evident at 4 days but the butter was putrid at 6 days and 7 days. The satisfactory sample did not contain coliform organisms, the total bacterial count was 2 per ml. on nutrient agar and 45 per ml. on T.G.E.M. agar, the proteolytic and lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not recovered. When the sample was used to wash experimental unsalted butter no significant flavor

defects were evident at 7 days.

Plant 70

Both samples of water from the well at plant 70 were unsatisfactory (table 70). Coliform organisms were present in the 50 ml. portion of each sample, total bacterial counts were 6 and 7 per ml. on nutrient agar and 125 and 150 per ml. on T.G.E.M. agar, proteolytic counts were 100 and 2 per ml. lipolytic counts did not exceed 1 per ml. and Ps. putrefaciens was not isolated. Experimental unsalted butter washed with one sample showed no noticeable flavor deterioration at 7 days, while butter washed with the other sample had an unclean flavor at 3 days.

The two samples of water that had been through a wooden storage tank also were unsatisfactory. Coliform organisms were present in the 50 ml. portion of one sample, total bacterial counts were 4 and 200 per ml. on nutrient agar and 340 and 350 per ml. on T.G.E.M. agar, proteolytic counts were 100 and 35 per ml., lipolytic counts did not exceed 2 per ml. and Ps. putrefaciens was not detected. Experimental unsalted butter washed with one sample showed no noticeable flavor deterioration at 4 days but was rancid at 6 days, while butter washed with the other sample was rancid at 4 days.

Table 70

Results on samples of water from plant 70

Sample no.	Type sample	Date examined	Test for *Escherichia-Aerobacter* species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	*P.	#L.	Test for *Ps. putrefaciens*	Action in experimental unsalted butter at 21°C.
1.	well	1-13-42	/ 50 ml.	6	125	100	<1	-	O.K. 7 da.
2.	"tank	1-13-42	/ 50 ml.	4	350	100	2	-	rancid 6 da.
3.	well	4-21-42	/ 50 ml.	7	150	2	1	-	unclean 3 da.
4.	tank	4-21-42	-	200	340	35	1	-	rancid 4 da.

*P. = proteolytic
#L. = lipolytic
"tank = wooden tank

Coliform Organisms in Creamery Water Samples

Distribution of coliform organisms in water from wells, storage tanks and municipal supplies

A number of the samples of water from wells, storage tanks and municipal supplies contained coliform organisms (table 71).

Of 224 well samples from 64 plants, 132 samples from 38 plants having satisfactory supplies did not contain coliform organisms. Of the 92 samples from 26 plants having unsatisfactory supplies, 54 samples from 22 plants showed coliform organisms; 21 samples showed the organisms only in the 50 ml. portion, 25 in the 50 ml. and 10 ml. portions and 8 in the 50 ml., 10 ml. and 1 ml. portions. The 54 positive samples included 24 per cent of the 224 well samples, and the 22 plants from which they came comprised 34 per cent of the 64 plants represented by the well samples or 85 per cent of the 26 plants having wells considered unsatisfactory.

The 185 tank samples came from 55 creameries. The 28 samples from 9 plants having satisfactory wells and tanks showed no coliform organisms. There were 24 plants which had satisfactory wells but were storing water in unsatisfactory tanks. Of 80 samples from these tanks, 25 showed coliform organisms; 13 showed the organisms in only the 50 ml. portion, 10 in the 50 ml. and 10 ml. portions and 2 in the 50 ml., 10 ml. and 1 ml. portions. The 25 positive samples included 14

Table 71

Distribution of coliform organisms in water from wells, supply tanks and municipal supplies

Type of sample	No. samples	No. plants represented	No. samples containing coliform organisms				No. plants represented by samples containing coliform organisms
			in 50 ml.	in 50,10 ml.	in 50,10,1 ml.	total	
Well							
satisfactory well	132	38	0	0	0	0	0
unsatisfactory well	92	26	21	25	8	54	22
Tank							
satisfactory well and tank	28	9	0	0	0	0	0
satisfactory well-unsatisfactory tank	80	24	13	10	2	25	14
unsatisfactory well and tank	77	22	21	16	9	46	17
City	27	11*	5	1	0	6	3[a]

* Including two plants using city water entirely.
[a] Including one plant using city water entirely.

per cent of the 185 tank samples, and the 14 plants from which they came made up 25 per cent of the 55 plants represented by the tank samples or 58 per cent of the 24 plants having unsatisfactory tanks but satisfactory wells. Of the 77 tank samples from 22 plants having unsatisfactory wells, 46 samples from 17 plants showed coliform organisms; 21 samples showed the organisms only in the 50 ml. portion, 16 in the 50 ml. and 10 ml. portions and 9 in the 50 ml., 10 ml. and 1 ml. portions. The 46 positive samples comprised 25 per cent of the 185 tank samples and the 17 plants from which they came made up 31 per cent of the 55 plants from which tank samples came or 77 per cent of the 22 plants using unsatisfactory wells.

Of the 27 samples from 11 plants using city water (two used only city water) 6 samples from 3 plants, one of which used city water entirely, showed coliform organisms; 5 samples showed the organisms only in the 50 ml. portion and one in the 50 ml. and 10 ml. portions. The 6 positive samples included 22 per cent of the 27 city water samples.

Distribution of coliform organisms in water and in experimental butter washed with the water

In eight trials experimental butter made in the usual way and washed with water samples known to contain coliform organisms was examined for the presence of these organisms, about 24 hours after it churned. In two trials the organisms were

present in 50 ml., in three trials they were present in 50 ml. and 10 ml. and in three trials they were present in 50 ml., 10 ml. and 1 ml. In each trial a portion of the butter remained unsalted while to the other portion 1.5 per cent salt was added. The presence of coliform organisms in butter was determined by inoculation of 1 ml. and 0.1 ml. portions of melted butter into tubes containing 10 ml. of standard lactose broth. To the tubes showing liberation of gas after 24 and 48 hours, the "completed test", as listed in "standard methods" (83), was applied. The results are shown in table 72.

In unsalted butter washed with the contaminated water, coliform organisms were present in 1 ml. in eight trials and in seven of the eight trials they were present in 0.1 ml. In salted butter the organisms were present in 1 ml. in four trials and in 0.1 ml. in one trial. Evidently the coliform organisms in water are more widely distributed in unsalted butter washed with the water than in salted butter.

There was no close correlation between the distribution of the organisms in the water and in the butter; in trial 1 the water contained coliform organisms in 1 ml. and the unsalted butter contained the organisms in 1 ml. but not in 0.1 ml., while in trial 3 the water showed coliform organisms only in 50 ml. but the unsalted butter showed the organisms in 1 ml. and 0.1 ml.

Table 72

Distribution of coliform organisms in water and in experimental butter washed with the water

Trial no.	Test for coliform organisms in water			Test for coliform organisms in butter			
				Unsalted		Salted*	
	50 ml.	10 ml.	1 ml.	1 ml.	0.1 ml.	1 ml.	0.1 ml.
1.	≠	≠	≠	≠	-	-	-
2.	≠	≠	≠	≠	≠	≠	-
3.	≠	-	-	≠	≠	-	-
4.	≠	≠	-	≠	≠	≠	≠
5.	≠	≠	≠	≠	≠	≠	-
6.	≠	-	-	≠	≠	≠	-
7.	≠	≠	-	≠	≠	-	-
8.	≠	≠	-	≠	≠	-	-

* On basis of 1.5 per cent added to the butter.

Distribution of coliform organisms in commercial butter

Thirty-two samples of commercial butter were examined, within 48 hours after churning, for the presence of coliform organisms, using 1 ml., 0.1 ml. and 0.01 ml. amounts. Six samples were unsalted, while 26 were salted. In each case the quality of the water used by the manufacturing plant was known.

With three samples of unsalted butter washed with water not containing coliform organisms, the organisms were not found in 1 ml., 0.1 ml. or 0.01 ml. All three samples of unsalted butter washed with water containing coliform organisms showed the organisms in 1 ml. two also showed the organisms in 0.1 ml. and one contained the organisms in 0.01 ml.

With 15 samples of salted butter that had been washed with water not containing coliform organisms, the organisms were not found in 13 instances and were found in two instances; one showed the organisms only in 1 ml., while the other showed the organisms in 1 ml. and 0.1 ml. The positive samples presumably were caused by contamination from sources other than wash water. With 11 samples of salted butter that had been washed with water containing coliform organisms, the organisms were found in 6 cases and not found in 5 cases. All 6 positive samples showed the organisms in 1 ml.; two also showed the organisms in 0.1 ml. The 5 negative samples presumably were due to the inhibitory effect of the salt.

Relationship between distribution of coliform organisms in water and total bacterial counts on the water

The relationship between the distribution of coliform organisms in water and the total bacterial counts on the water was tested with the first 385 samples as follows: The samples were divided into four groups, (a) those showing no coliform organisms in 50 ml., (b) those showing the organisms in 50 ml., (c) those showing the organisms in 50 ml. and 10 ml. and (d) those showing the organisms in 50 ml. 10 ml. and 1 ml. For each group the total bacterial counts (on T.G.E.M. agar, 96 hours at 21°C.) were transferred to logarithms (a count of less than 1 was considered as 1) and the mean logarithm and corresponding number for each group were obtained (table 73)*.

There are significant differences among the mean logarithms for the groups. Clearly, the outstanding difference is that between group a and the other groups, but the differences between groups b, c and d are of considerable magnitude. The trend upwards from group b to group d is almost exactly linear. The data show that as the coliform organisms become more numerous in water, the total bacterial counts on the water tend to increase.

* The calculations were made in the Statistical Laboratory under the direction of Prof. G. W. Snedecor.

Table 73

Relationship between the distribution of coliform organisms in water and the total bacterial counts on the water*

Group	Water samples: Coliform organisms in 50 ml.	10 ml.	1 ml.	No. samples	Mean logarithm of total bacterial counts	Corresponding total bacterial count
a.	-	-	-	273	1.537	34
b.	✓	-	-	50	2.289	195
c.	✓	✓	-	43	2.447	280
d.	✓	✓	✓	19	2.645	442

* Total bacterial counts on T.G.E.M. agar after 96 hours at 21°C.

Comparison of standard lactose broth and tryptose lauryl-sulfate broth as presumptive media for detecting coliform organisms in water

The data included in tables 1 to 70 relative to the presence of coliform organisms in creamery water supplies were obtained by use of standard lactose broth as the presumptive medium. Plantings of the samples in tryptose lauryl-sulfate broth also were made. The comparative results obtained with the two media are given in table 74; only the tests with 50 ml. portions of water are considered.

With the 436 samples of water examined, standard lactose broth gave 147 positive presumptive tests, of which 131 tests (89.1 per cent) were completely confirmed. With the same samples, tryptose lauryl-sulfate broth gave 138 positive presumptive tests, of which 131 tests (94.9 per cent) were completely confirmed. The completely confirmed tests included two samples with which the lactose broth tests were positive, while the tryptose broth tests were negative; on the other hand, two samples gave negative tests with lactose broth and positive tests with tryptose broth. With 129 samples, both the lactose broth and tryptose broth tests were positive. The 16 non-confirming positive presumptive tests with lactose broth and the 7 with tryptose broth included 5 samples which gave positive tests with both media, 11 samples which gave positive tests with lactose broth and negative tests with tryptose broth and 2 samples which gave negative tests with

Table 74

Comparison of standard lactose broth and tryptose lauryl-sulfate broth as presumptive media for detecting coliform organisms in water

Medium	No. samples examined (50 ml.)	No. positive presumptive tests	Positive presumptive tests confirmed		No. positive presumptive tests not confirmed
			No.	Per cent	
Standard lactose broth	436	147	131*	89.1	16[a]
Tryptose lauryl-sulfate broth	436	138	131*	94.9	7[a]

* Includes 2 lactose broth ≠ tryptose broth -
2 lactose broth - tryptose broth ≠
129 lactose broth ≠ tryptose broth ≠

[a] Includes 11 lactose broth ≠ tryptose broth -
2 lactose broth - tryptose broth ≠
5 lactose broth ≠ tryptose broth ≠

lactose broth and positive tests with tryptose broth.

Identification of coliform organisms found in some Iowa creamery water supplies

During the examination of one sample of water from the supply of each of 70 creameries, 51 isolations of coliform organisms were made. Each isolation represented the main colony type appearing on an eosin methylene-blue agar plate streaked from lactose broth showing gas formation after inoculation with 50 ml. of water.

Using Bergey's 1939 system of classification (1), the 51 cultures were identified as follows: 12 cultures (24 per cent) as Escherichia coli, 12 cultures (24 per cent) as Escherichia freundii, 16 cultures (31 per cent) as Aerobacter aerogenes and 11 cultures (21 per cent) as Aerobacter cloacae.

A large number of other coliform cultures were isolated but not identified. Presumably those obtained during a single examination of each supply were representative of the total number of samples studied.

Comparison of Average Total Bacterial Counts Made Under Various Conditions

Total bacterial counts on 436 samples of water were obtained under three sets of conditions; with each set two incubation periods were employed. The detailed conditions used were (a) nutrient agar at 37°C. for 24 and 48 hours, (b) T.G.E.M. agar at 37°C. for 24 and 48 hours and (c) T.G.E.M.

agar at 21°C. for 48 and 96 hours.

Examination of data included in tables 1 to 70 suggest that, in general, T.G.E.M. agar counts at 37°C. were higher than corresponding nutrient agar counts at 37°C., total counts on T.G.E.M. agar after 48 hours at 37°C. were higher than those on the same medium after 48 hours at 21°C. and counts on T.G.E.M. agar after 96 hours at 21°C. were the highest of any of the counts obtained. However, some variations from the general relationships were evident in that certain nutrient agar counts were higher than corresponding counts on T.G.E.M. agar under the same incubation conditions, certain T.G.E.M. agar counts after 48 hours at 21°C. were higher than the corresponding T.G.E.M. agar counts after 48 hours at 37°C. and certain T.G.E.M. agar counts after 96 hours at 21°C. were not higher than other counts obtained.

The total bacterial counts were transferred to logarithms (a count of less than 1 was considered as 1) and the mean logarithm and corresponding number were obtained for each of the plating conditions (table 75).

The average count on T.G.E.M. agar at 37°C. was higher than on nutrient agar, with both 24 and 48 hour incubation periods. The average count on T.G.E.M. agar after 48 hours at 37°C. was higher than on T.G.E.M. agar after 48 hours at 21°C. The average count on T.G.E.M. agar after 96 hours at 21°C. was the highest obtained with any of the conditions employed and presumably included various slower-growing

Table 75

Average total bacterial counts on 436 samples of water plated under various conditions

Average bacterial counts obtained by logarithms*

Medium	Incubation		Average total bacterial count
	temp.	time	
nutrient agar	37°C.	24 hrs.	4
		48 hrs.	11
T.G.E.M. agar	37°C.	24 hrs.	6
		48 hrs.	21
	21°C.	48 hrs.	11
		96 hrs.	63

* Milk ordinance and code. U. S. Public Health Service Bul. 220. 1939.

organisms found in water.

Organisms of the Genus Pseudomonas in Creamery Water Supplies

Relationship of presence of Ps. putrefaciens in water to presence of coliform organisms, to total and proteolytic counts and to spoilage in unsalted butter

Of 436 water samples from the 70 plants, 22 samples, representing 14 plants, yielded Ps. putrefaciens (table 76). The positive samples included 6 well samples from 5 plants, 12 supply tank samples from 9 plants and 4 city water samples from 3 plants; of the supply tanks involved in the 12 samples, some were wood and some were metal. In the case of plant 49, two of the four well samples and three of the four supply tank samples yielded Ps. putrefaciens; one negative well sample and the one negative tank sample were collected on the same day and presumably represent a period when the water was free of the organisms. The other water supplies yielding Ps. putrefaciens contained the organisms only in a relatively small percentage of the samples.

Of the 22 samples yielding Ps. putrefaciens, 6 (27 per cent) did not show coliform organisms in 50 ml., 4 (18 per cent) contained them in the 50 ml. portion, 7 (32 per cent) contained them in the 50 ml. and 10 ml. portions and 5 (23 per cent) contained them in the 50 ml., 10 ml. and 1 ml. portions. Eighteen (82 per cent) of the 22 samples had total bacterial counts on T.G.E.M. agar after 96 hours at 21°C.

Table 76

Data on water samples yielding Ps. putrefaciens

Plant no.	Source of sample	Test for Escherichia-Aerobacter species	Bacteria per ml. 24 hrs. at 37°C. on nutrient agar	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Total	Bacteria per ml. 96 hrs. at 21°C. on T.G.E.M. agar Proteolytic	Action in experimental unsalted butter at 21°C.
5.	wooden tank	-	15	1000	300	putrid 4 da.
11.	city	-	<1	55	3	O.K. 7 da.
16.	wooden tank	-	<1	15	3	musty 4 da.
17.	metal tank	⁄50 ml.	<1	17	3	O.K. 7 da.
29.	wooden tank	-	42	400	40	putrid 3 da.
32.	city	-	13	125	2	putrid 4 da.
38.	well B	⁄50,10 ml.	5	400	10	rancid 7 da.
	wooden tank	⁄50,10 ml.	55	800	80	rancid 7 da.
41.	well	-	2	11	2	putrid 4 da.
46.	city	⁄50 ml.	1	260	39	rancid 7 da.
	city	⁄50,10 ml.	28	210	60	rancid 7 da.
	well	⁄50,10 ml.	120	375	125	off 7 da.
49.	well	⁄50,10,1 ml.	80	1200	100	putrid 4 da.
	well	⁄50,10,1 ml.	140	700	5	off 3 da.
	metal tank	⁄50,10,1 ml.	20	700	4	putrid 4 da.
	metal tank	⁄50,10,1 ml.	170	1000	100	putrid 2 da.
	metal tank	⁄50,10,1 ml.	150	550	6	off 3 da.
54.	well	⁄50,10 ml.	6	700	100	unclean 7 da.
62.	wooden tank	⁄50 ml.	3	4200	300	putrid 2 da.
64.	wooden tank	⁄50,10 ml.	200	1000	200	putrid 2 da.
	wooden tank	⁄50,10 ml.	70	650	10	unclean 6 da.
69.	metal tank	⁄50 ml.	16	130	16	putrid 7 da.

in excess of 100 per ml., while only 5 (23 per cent) had total bacterial counts on nutrient agar after 24 hours at 37°C. in excess of 100 per ml. Proteolytic counts on the 22 samples ranged from 2 to 300 per ml.; of these counts 6 (27 per cent) were less than 5 per ml.

Nineteen (86 per cent) of the 22 samples yielding Ps. putrefaciens produced spoilage to some degree when portions of experimental unsalted butter were washed with them and held at 21°C. for 7 days. Of the 19 lots of butter showing spoilage, 10 were putrid, 4 were rancid, 2 had an unclean flavor and 3 were criticized as being off in flavor with no particular defect conspicuous enough to be cited. One of the 22 lots of butter was musty at 4 days, while two showed no defect even at 7 days; the water samples used to wash three lots of butter comprised three (75 per cent) of the four samples of water having total bacterial counts on T.G.E.M. agar of less than 100 per ml. In 11 (58 per cent) of the 19 lots of butter showing spoilage the defect was evident at 4 days or earlier.

Relationship of presence of various Pseudomonas species in wash water to spoilage in experimental unsalted butter

Certain Pseudomonas species are known to be relatively abundant in water and to reveal their presence through characteristic fluorescent colonies on agar plates and through undesirable flavors in dairy products contaminated with the

water.

Of 436 water samples from the 70 plants, 105 samples representing 23 plants gave typical fluorescent colonies on T.G.E.M. agar plates held at 21°C. for 96 hours. At present these would be considered colonies of Pseudomonas fluorescens or closely related species, but Garrison (17) has shown that identification is not feasible using the present descriptions of the organisms (1).

Of the 105 water samples containing fluorescent bacteria, 97 (92 per cent) produced flavor defects in experimental unsalted butter held at 21°C. for 7 days. The 97 samples showing flavor deterioration included 42 (43 per cent) that were rancid, 25 (26 per cent) showing a putrid condition, 20 (21 per cent) that were cheesy and 10 (10 per cent) that were criticized as unclean, off or otherwise defective. Seventy-one (73 per cent) of the 97 butter samples showed one or more defects at 4 days or earlier. In a number of samples, more than one flavor was evident when the butter was criticized, with different defects becoming more prominent as the holding period progressed. Only 25 (60 per cent) of the 42 samples that were rancid at 7 days were noticeably rancid at 4 days or earlier. On the other hand 46 (84 per cent) of the 55 samples that showed a cheesy, putrid or unclean flavor at 7 days were defective at 4 days or earlier.

From three samples of experimental unsalted butter showing a fruity or rancid odor, organisms identified as

Pseudomonas fragi were easily isolated. Although no particular effort was made to isolate these organisms from the various samples of water, it is probable that a number of them contained the organisms since litmus milk tubes inoculated with water and held at approximately 3°C. sometimes gave a typical May apple odor.

When experimental unsalted butter was washed with one sample of water and held at 21°C., a potato odor was evident at 4 days. An organism resembling Pseudomonas graveolens (62) was isolated. Sterile water inoculated with a pure culture and used to wash a portion of experimental unsalted butter reproduced the potato odor.

In one lot of experimental unsalted butter held at 21°C. a skunk-like odor was noted at 4 days. After considerable difficulty, an organism resembling Pseudomonas mephitica (6) was isolated; it reproduced the skunk-like odor in butter when a pure culture was used to inoculate sterile wash water.

General Consideration of Significance of Total Bacterial Counts on Creamery Water Supplies

With the variety of types of bacteria encountered in creamery water supplies, two samples of water having essentially the same numbers of bacteria per ml. might have very different effects on butter. Certain types of organisms, such as the fluorescent bacteria, are known to be generally

capable of causing serious flavor deterioration in butter, while many other types are not believed to be concerned with butter deterioration. Some organisms, such as Ps. putrefaciens, apparently may bring about a putrid condition in butter when present initially in small numbers. Since this species frequently fails to grow on the media commonly used it may be present in water without being included in the total bacterial count.

The effects on butter keeping quality of 181 samples of water having total bacterial counts of 100 or more (on T.G.E.M. agar, 96 hours at 21°C.) are summarized in table 77; the samples are divided into six groups on the basis of the bacterial counts.

The percentages of the samples that produced no flavor deterioration in butter at 4 days increased from group 1 to group 2 but decreased from then on throughout the remaining groups; the maximum percentage was 63 for group 2 and the minimum was 36 for group 6. When the data are considered from the standpoint of no production of flavor defects at 7 days there was the same general trend in the percentages but the values were smaller; the maximum percentage was 37 for group 2 and the minimum was 9 for group 6.

While some samples of water having high bacterial counts did not cause flavor deterioration when used to wash experimental unsalted butter even at 7 days, the tendency was for water samples showing increased counts to be more generally

Table 77

Relationship of total bacterial counts on water to deterioration in experimental unsalted butter washed with the water

Bacterial counts: Groups	Bacterial counts: Range per ml.	No. samples in group	Per cent of samples in group: producing no flavor deterioration at 4 days	Per cent of samples in group: producing no flavor deterioration at 7 days
1.	100 to 200	52	54	35
2.	201 to 300	27	63	37
3.	301 to 400	22	50	23
4.	401 to 750	33	48	18
5.	751 to 1500	36	39	11
6.	over 1500	11	36	9

associated with greater deterioration in the butter.

Chlorination Studies on Some Samples of Iowa Creamery Water

In the studies on the effect of chlorination on Iowa creamery water, a number of plants having unsatisfactory supplies were selected and samples of water obtained in them.

To a 250 ml. volume of each of 30 samples of water from the supplies of 16 Iowa creameries were added 2.5 ml., 12.5 ml. and 25 ml. of a hypochlorite solution made up from B-K powder and containing 100 ppm. of chlorine. The amounts added were expected to give a chlorine content of approximately 1, 5 and 10 ppm., respectively, to the sample. The chlorine content was determined immediately after the addition of the hypochlorite solution to the water, using the starch iodide titration listed in "standard methods" (83) on a 50 ml. portion, and again after a holding period of 15 minutes at approximately 10°C. Total bacterial counts, the presence of coliform organisms and the presence of Ps. putrefaciens were determined on the unchlorinated and the chlorinated water. In order to stop the action of the chlorine at the completion of the holding period, 5 ml. portions of chlorinated water were transferred to dilution bottles containing 45 ml. of sterile water and an amount of sodium thiosulfate just in excess of that required to destroy the chlorine present. Duplicate 10 ml. and 1 ml. portions of the resulting 1:10

dilution were added to lactose broth tubes of suitable concentration to test for the presence of coliform organisms. Duplicate 1 ml. portions of the 1:10 dilution were plated to determine the numbers of bacteria. Duplicate 3 ml. and 1 ml. portions of chlorinated water were added to sterile litmus milk tubes; the tubes were held at approximately 3°C. and examined at the end of 2 and 4 weeks for the presence of Ps. putrefaciens. Ps. putrefaciens was not found in any of the original samples so results with reference to it are not given.

The data on 16 of the 30 samples are presented in Table 78. The 16 samples included 13 from supply tanks, 2 from wells and 1 from a municipal supply. The other 14 samples were well samples from 14 of the 16 plants; except that the bacterial counts on the water before chlorination were lower, the 14 samples gave essentially the same results as the 16 samples and detailed results are not included.

Six of the 16 samples originally contained coliform organisms in 1 ml. amounts. Additions of approximately 1, 5 and 10 ppm. of chlorine to the samples, with holding for 15 minutes at approximately 10°C., eliminated coliform organisms from the volumes of water studied. The chlorine treatment greatly reduced the numbers of bacteria present in all samples except 49T which contained relatively large numbers of aerobic sporeformers; these were the organisms surviving treatment even with 10 ppm. of chlorine. With all except sample 6T,

Table 78

Chlorination studies on some samples of Iowa creamery water

Sample no.	Chlorine content		Test for coliform organisms	Bacteria per ml.	
	initial (ppm.)	final* (ppm.)		after 48 hrs. at 37°C.	after 96 hrs. at 21°C.
6T**	--	--	-[a]	150	180
	.91	.91	-	<10	<10
	4.12	1.83	-	<10	<10
	9.06	6.95	-	<10	<10
9T	--	--	≠ 1 ml.[a]	100	350
	.98	.98	-	<10	<10
	4.91	4.70	-	<10	<10
	10.18	10.11	-	<10	<10
18T	--	--	-	50	375
	.98	.98	-	<10	<10
	4.63	4.42	-	10	20
	9.69	9.41	-	<10	<10
25T	--	--	-	350	600
	.98	.91	-	10	<10
	5.12	4.84	-	<10	<10
	10.39	9.98	-	<10	<10
26T	--	--	-	13	45
	1.12	.98	-	<10	<10
	4.56	4.14	-	<10	<10
	9.41	8.21	-	<10	<10
36T	--	--	≠ 1 ml.	50	400
	1.05	.91	-	<10	<10
	5.41	5.12	-	<10	<10
	10.95	10.53	-	<10	<10
40T	--	--	-	1800	1500
	1.05	1.05	-	10	20
	4.91	4.91	-	<10	<10
	10.60	10.53	-	10	<10
41T	--	--	-	190	180
	.91	.91	-	20	<10
	4.91	4.77	-	<10	<10
	10.53	10.39	-	<10	<10

Table 78 (continued)

Sample no.	Chlorine content		Test for coliform organisms	Bacteria per ml.	
	initial (ppm.)	final* (ppm.)		after 48 hrs. at 37°C.	after 96 hrs. at 21°C.
42W	--	--	-	260	320
	.84	.84	-	<10	<10
	5.61	4.77	-	<10	<10
	9.77	9.56	-	<10	<10
48T	--	--	-	300	350
	.84	.84	-	<10	<10
	4.77	4.56	-	<10	<10
	9.84	9.34	-	<10	<10
49T	--	--	≠ 1 ml.	1700	1400
	.98	.84	-	400	10
	5.12	5.05	-	40	50
	10.46	10.26	-	30	30
50C	--	--	-	3	18
	.98	.98	-	<10	<10
	5.05	4.49	-	<10	<10
	9.91	9.20	-	<10	<10
51T	--	--	≠ 1 ml.	600	1200
	1.05	.98	-	<10	10
	5.54	5.05	-	10	<10
	11.16	11.02	-	<10	<10
52W	--	--	≠ 1 ml.	150	90
	.98	.91	-	<10	<10
	4.91	4.63	-	<10	<10
	10.67	9.98	-	<10	<10
64T	--	--	≠ 1 ml.	1200	1800
	1.05	.98	-	<10	<10
	4.98	4.91	-	<10	<10
	9.75	9.75	-	<10	<10
70T	--	--	-	260	350
	.98	.91	-	<10	<10
	4.84	4.34	-	<10	<10
	10.19	9.62	-	<10	<10

* After 15 minutes at approximately 10°C.
** T = tank, W = well, C = city
[a] Duplicate 1 and 0.1 ml. portions employed.

there was relatively little difference between the initial and the final chlorine contents; with this sample and also with the well sample from plant 6 there was considerable decrease in the chlorine during the holding. However, even with the decrease, satisfactory reductions of numbers of bacteria were obtained.

DISCUSSION

Results of the examination of samples of water from Iowa creamery supplies show that some of them need improvement. This situation obtains when the supplies are considered from the standpoint of public health and is equally evident when they are considered from the standpoint of butter manufacture. Water containing coliform organisms is potentially dangerous and is definitely undesirable for human consumption, directly, or indirectly through the medium of a food product. Water containing organisms capable of causing spoilage in butter is serious from an economic standpoint.

The data show that coliform organisms present in water used to wash butter may carry over to the finished butter and that, while the presence of salt has a definitely inhibitory action on the organisms, some of them may survive. This relationship is in agreement with the results of Hammer and Yale (23). It clearly indicates that from a public health standpoint, and from the standpoint of prevention of butter spoilage, some of the value of efficient pasteurization of cream and of other sanitary precautions is nullified if contaminated wash water is used in a plant.

In general, the total bacterial counts obtained on T.G.E.M. agar after 96 hours at 21°C. tended to increase as

coliform organisms became more numerous in water. However, some water samples containing coliform organisms in the smallest portion tested (1 ml.) showed comparatively low total bacterial counts. No such general relationship appeared to exist between the distribution of coliform organisms in water and total bacterial counts on nutrient agar after 24 hours at 37°C. Riddick (72) suggested that coliform bacteria are associated with high total bacterial counts on water samples and that the total count can be used as an index to control the incidence of the intestinal group of organisms in water.

The inhibition of coliform organisms in some water samples by selective media, when the organisms have been shown present by a completely confirmed standard lactose broth test, has sometimes resulted in the discarding of such selective media. According to the data obtained, the number of water samples containing coliform organisms that were "missed" by the tryptose lauryl-sulfate broth test was comparatively small and was balanced by an equal number that were "missed" by the lactose broth test but "picked up" by the tryptose broth test. Mallmann and Darby (48) and Levine (39) have shown the value of the latter test in reducing the number of false presumptive tests encountered in water analysis. Apparently further study along this line is advisable; such data will accumulate with more widespread use of the medium in routine analysis.

It appears that the change from nutrient agar to T.G.E.M.

agar for the determination of numbers of organisms present in milk could advantageously be extended to water analysis. Due to the variety of nutrients present in T.G.E.M. agar, the numbers of organisms would be expected to be higher with it than with nutrient agar under the same conditions of incubation. The larger counts obtained with the medium give a more complete picture of the numbers of organisms actually present in a sample of milk or water. Additional comparative studies would appear to be of value, continuing the use of the 37°C. incubation temperature and including 24 and 48 hours incubation periods. The 37°C. count is of value especially from the public health standpoint; a 21°C. count is advisable where information is required concerning the value of a water supply for use in a butter plant since certain species not particularly significant from a public health standpoint grow better at the lower temperature.

Although water containing coliform organisms may not cause serious flavor defects in butter, the fact that other undesirable organisms usually are present in water along with coliform organisms makes its use inadvisable, entirely aside from the public health standpoint. However, water not containing coliform organisms often contains certain undesirable species capable of producing defects in butter, and it is for this reason that the usual public health analysis does not suffice; most of these undesirable organisms, particularly those belonging to the genus Pseudomonas, grow

better at relatively low temperatures. In this connection the total bacterial count on T.G.E.M. agar after 96 hours at 21°C. is of added value in that it not only gives an indication of total numbers of organisms present in water but also furnishes information on types of organisms present.

The fact that a sample of water contains relatively large numbers of organisms does not signify that butter will spoil after being washed with the water. To produce spoilage, the organisms must be capable of causing objectionable changes in the presence of certain inhibiting factors, such as salt or acid, or after dispersion of the moisture and nutrients. However, water showing high total, proteolytic and lipolytic counts commonly will contain a variety of bacteria and usually will include some of the more harmful species. The occurrence of fluorescent organisms, whose relationship to butter spoilage is well established, in approximately 25 per cent of all the water samples examined, including some of relatively low counts, is sufficient evidence of the need for adequate control of water supplies.

From the public health angle and from the standpoint of the butter manufacturer, the question is inevitably raised, what can be done to improve water supplies?

There are a number of possibilities offering considerable promise. New wells could be constructed at some plants, but there is no quarantee that the quality of water obtained would be satisfactory. Repair of wells is difficult.

Sterilization of wells has been practiced with apparent success (80). In some plants the supply tanks have been eliminated. This was possible in certain plants in the section of Iowa covered, but it would not be feasible everywhere and would be of value only where the well water was of satisfactory quality. Frequent cleaning of tanks should reduce the contamination of well water in supply tanks and must be followed where the use of a supply tank is considered to be necessary. The use of water tank coatings designed to give no odors or tastes to stored water requires investigation. In most cases, and particularly in butter manufacturing plants where water is tempered in a tank or vat, it appears that the treatment of such water by chlorine is the most economical and practicable method available. The variable quality of some of the wells studied emphasizes the need for some type of treatment.

Chlorination is in use in many cities for treatment of municipal water supplies and has given consistently satisfactory and reliable results. It has been demonstrated that water containing fairly large concentrations of chlorine (up to 25 ppm.) causes no noticeable off-flavors when used to wash butter (27). The concentration of residual chlorine in municipal water supplies, while sufficient to kill pathogenic and coliform organisms, needs to be studied from the standpoint of species undesirable in butter manufacture, particularly those of the genus Pseudomonas. Most sporeforming

organisms probably are not appreciably affected by concentrations up to 10 ppm., but they are not often involved in butter spoilage since they probably grow very little, if at all, at the temperatures ordinarily used for transportation and storage of butter. Although the questions of extreme dissipation of chlorine by some types of water and possible chlorine resistance of certain organisms must be kept in mind, it appears that water to be used to wash butter can be satisfactorily treated by addition of 1 to 5 ppm. of an active hypochlorite solution and holding for 15 minutes at the washing temperature. Since hypochlorite solutions are in use in many dairy plants, the development of satisfactory procedures for chlorination of creamery water supplies should not be difficult.

SUMMARY AND CONCLUSIONS

1. An examination of 436 unchlorinated water samples from 70 butter plants, using the usual types of bacteriological tests and a few additional ones, indicated that some plant supplies regularly were satisfactory, some regularly were unsatisfactory while others varied in quality from one examination to another. Certain supplies that would have been considered acceptable from a public health standpoint were not suitable for use in butter manufacture. Over one-half the plant wells regularly gave satisfactory water, but many of these supplies were contaminated in storage tanks. Various city water supplies were unsatisfactory for butter manufacturing purposes.

2. Coliform organisms regularly were absent from some plant supplies (well and tank), regularly were present in others and sometimes were found in still others. Two-thirds of the plant wells regularly were free of coliform organisms in the volumes examined, but in many cases the organisms were being added in storage tanks. Some of the city water supplies contained coliform organisms.

3. When experimental butter was washed with water known

to contain coliform organisms, the organisms regularly were found in 1 ml. of unsalted butter and usually were found in 0.1 ml.; they sometimes were found in these amounts of salted butter. Commercial butter, from plants using water that commonly contained coliform organisms, regularly contained the organisms when unsalted and sometimes contained them when salted.

4. As coliform organisms became more numerous in water, total bacterial counts on the water tended to be higher.

5. The use of tryptose lauryl-sulfate broth on the 436 water samples reduced somewhat the number of spurious presumptive tests, in comparison with the use of standard lactose broth.

6. The average total bacterial count, after 24 or 48 hours at 37°C., was higher on T.G.E.M. agar than on nutrient agar. The average T.G.E.M. agar count after 48 hours at 37°C. was higher than that after 48 hours at 21°C., but the average T.G.E.M. agar count after 96 hours at 21°C. was the highest count obtained.

7. Approximately 5 per cent of the water samples yielded Ps. putrefaciens; most of the positive samples contained coliform organisms, had total counts (T.G.E.M. agar, 96 hours, 21°C.) in excess of 100 per ml. and produced spoilage in experimental unsalted butter.

8. Approximately one-fourth of the water samples contained typical fluorescent organisms; most of the positive

samples produced spoilage in experimental unsalted butter.

9. Some Pseudomonas species known to produce undesirable flavors in butter were isolated from certain water samples; these included Ps. fragi, Ps. graveolens and Ps. mephitica.

10. While some samples having high bacterial counts did not cause flavor deterioration when used to wash experimental unsalted butter, the tendency was for samples showing high counts to be more generally associated with serious deterioration in butter than samples having low counts.

11. Addition of 1, 5 or 10 ppm. of chlorine to samples of contaminated water, with holding at approximately 10°C. for 15 minutes, resulted in satisfactory destruction of non-sporeforming organisms. Apparently, sporeformong species were not appreciably affected.

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